```
Connecting via Winsock to STN


Welcome to STN International!  Enter x:x

LOGINID:ssspta1644pnh

PASSWORD:
TERMINAL (ENTER 1, 2, 3, OR ?):2


* * * * * * * * * *   Welcome to STN International   * * * * * * * * * *

 NEWS  1          Web Page URLs for STN Seminar Schedule - N. America
 NEWS  2          "Ask CAS" for self-help around the clock
 NEWS  3  FEB 25  CA/CAPLUS - Russian Agency for Patents and Trademarks
                  (ROSPATENT) added to list of core patent offices covered
 NEWS  4  FEB 28  PATDPAFULL - New display fields provide for legal status
                  data from INPADOC
 NEWS  5  FEB 28  BABS - Current-awareness alerts (SDIs) available
 NEWS  6  FEB 28  MEDLINE/LMEDLINE reloaded
 NEWS  7  MAR 02  GBFULL: New full-text patent database on STN
 NEWS  8  MAR 03  REGISTRY/ZREGISTRY - Sequence annotations enhanced
 NEWS  9  MAR 03  MEDLINE file segment of TOXCENTER reloaded
 NEWS  10 MAR 22  KOREAPAT now updated monthly; patent information enhanced
 NEWS  11 MAR 22  Original IDE display format returns to REGISTRY/ZREGISTRY
 NEWS  12 MAR 22  PATDPASPC - New patent database available
 NEWS  13 MAR 22  REGISTRY/ZREGISTRY enhanced with experimental property tags
 NEWS  14 APR 04  EPFULL enhanced with additional patent information and new
                  fields
 NEWS  15 APR 04  EMBASE - Database reloaded and enhanced
 NEWS  16 APR 18  New CAS Information Use Policies available online

 NEWS EXPRESS  JANUARY 10 CURRENT WINDOWS VERSION IS V7.01a, CURRENT
               MACINTOSH VERSION IS V6.0c(ENG) AND V6.0Jc(JP),
               AND CURRENT DISCOVER FILE IS DATED 10 JANUARY 2005

 NEWS HOURS    STN Operating Hours Plus Help Desk Availability
 NEWS INTER    General Internet Information
 NEWS LOGIN    Welcome Banner and News Items
 NEWS PHONE    Direct Dial and Telecommunication Network Access to STN
 NEWS WWW      CAS World Wide Web Site (general information)

Enter NEWS followed by the item number or name to see news on that
specific topic.

  All use of STN is subject to the provisions of the STN Customer
  agreement.  Please note that this agreement limits use to scientific
  research.  Use for software development or design or implementation
  of commercial gateways or other similar uses is prohibited and may
  result in loss of user privileges and other penalties.

  * * * * * * * * * * * * * * STN  Columbus  * * * * * * * * * * * * * * * *

FILE 'HOME' ENTERED AT 16:40:03 ON 18 APR 2005

=>  file medline embase biosis scisearch caplus
COST IN U.S. DOLLARS                                SINCE FILE      TOTAL
                                                       ENTRY    SESSION
FULL ESTIMATED COST                                     0.21       0.21

FILE 'MEDLINE' ENTERED AT 16:40:17 ON 18 APR 2005
```

FILE 'EMBASE' ENTERED AT 16:40:17 ON 18 APR 2005

FILE 'BIOSIS' ENTERED AT 16:40:17 ON 18 APR 2005

FILE 'SCISEARCH' ENTERED AT 16:40:17 ON 18 APR 2005

FILE 'CAPLUS' ENTERED AT 16:40:17 ON 18 APR 2005

=> s attenuated E coli
L1 31 ATTENUATED E COLI

=> dup remove l1
PROCESSING COMPLETED FOR L1
L2 9 DUP REMOVE L1 (22 DUPLICATES REMOVED)

=> d l2 1-9 cbib abs

L2 ANSWER 1 OF 9 CAPLUS COPYRIGHT 2005 ACS on STN
2004:371738 Document No. 141:294155 Multivalent Shigella/enterotoxigenic Escherichia coli vaccine. Barry, Eileen M.; Levine, Myron M. (University of Maryland School of Medicine, Baltimore, MD, USA). New Generation Vaccines (3rd Edition), 751-754. Editor(s): Levine, Myron M. Marcel Dekker, Inc.: New York, N. Y. ISBN: 0-8247-4071-8 (English) 2004. CODEN: 69FIV5.
AB A review on multivalent Shigella/enterotoxigenic Escherichia coli vaccine (ETEC). Topics discussed include strategy to achieve broad-spectrum protection against Shigella, Shigella harboring mutations in guaBA and in the genes encoding Shigella enterotoxins 1 and 2, antigenic diversity among human ETEC pathogens, infection-derived immunity to ETEC, lessons learned from studies with a prototype **attenuated E. coli** live oral ETEC vaccine, and multivalent Shigella-ETEC live vector vaccine.

L2 ANSWER 2 OF 9 CAPLUS COPYRIGHT 2005 ACS on STN
2004:260312 Document No. 141:271300 Effects of propofol on endotoxin-induced acute lung injury in rabbit. Kwak, Sang Hyun; Choi, Jeong Il; Park, Jong Tae (Department of Anesthesiology and Pain Medicine, Medical School, Chonnam National University, Gwangju, 501-757, S. Korea). Journal of Korean Medical Science, 19(1), 55-61 (English) 2004. CODEN: JKMSEH. ISSN: 1011-8934. Publisher: Korean Academy of Medical Science.
AB This study was undertaken to clarify the effects of propofol on endotoxin-induced acute lung injury. Rabbits were randomly assigned to one of four groups. Each group received i.v. infusion of saline only, saline and Escherichia coli endotoxin, propofol (1 mg/kg bolus, then 5 mg/kg/h) and endotoxin, or propofol (4 mg/kg bolus, then 20 mg/kg/h) and endotoxin resp. Infusion of saline or propofol was started 0.5 h before the infusion of saline or endotoxin, and continued for 6 h thereafter. The lungs of rabbits were ventilated with 40% oxygen. Mean blood pressure, heart rate, arterial oxygen tension (PaO2), and peripheral blood leukocyte and platelet count were recorded. The wet/dry (W/D) weight ratio of lung and lung injury score were measured, and anal. of bronchoalveolar lavage fluid (BALF) was done. Endotoxin decreased PaO2, and peripheral blood leukocyte and platelet count. And it increased W/D ratio of lung, lung injury score and leukocyte count, percentage of PMN cells, concentration of albumin, thromboxane B2 and IL-8 in BALF. Propofol attenuated all these changes except the leukocyte count in peripheral blood. In conclusion, propofol attenuated endotoxin-induced acute lung injury in rabbits mainly

by inhibiting neutrophil and IL-8 responses, which may play a central role in sepsis-related lung injury.

L2 ANSWER 3 OF 9 MEDLINE on STN DUPLICATE 1

2003204492. PubMed ID: 12724393. Molecular analysis of mutS expression and mutation in natural isolates of pathogenic Escherichia coli. Li Baoguang; Tsui Ho-Ching T; LeClerc J Eugene; Dey Manashi; Winkler Malcolm E; Cebula Thomas A. (Center for Food Safety and Applied Nutrition, Food and Drug Administration, Laurel, MD 20708, USA.) Microbiology (Reading, England), (2003 May) 149 (Pt 5) 1323-31. Journal code: 9430468. ISSN: 1350-0872. Pub. country: England: United Kingdom. Language: English.

AB Deficiencies in the MutS protein disrupt methyl-directed mismatch repair (MMR), generating a mutator phenotype typified by high mutation rates and promiscuous recombination. How such deficiencies might arise in the natural environment was determined by analysing pathogenic strains of Escherichia coli. Quantitative Western immunoblotting showed that the amount of MutS in a wild-type strain of the enterohaemorrhagic pathogen E. coli O157 : H7 decreased about 26-fold in stationary-phase cells as compared with the amount present during exponential-phase growth. The depletion of MutS in O157 : H7 is significantly greater than that observed for a laboratory-**attenuated E. coli** K-12 strain. In the case of stable mutators, mutS defects in strains identified among natural isolates were analysed, including two E. coli O157 : H7 strains, a diarrhoeagenic E. coli O55 : H7 strain, and a uropathogenic strain from the E. coli reference (ECOR) collection. No MutS could be detected in the four strains by Western immunoblot analyses. RNase T2 protection assays showed that the strains were either deficient in mutS transcripts or produced transcripts truncated at the 3' end. Nucleotide sequence analysis revealed extensive deletions in the mutS region of three strains, ranging from 7.5 to 17.3 kb relative to E. coli K-12 sequence, while the ECOR mutator contained a premature stop codon in addition to other nucleotide changes in the mutS coding sequence. These results provide insights into the status of the mutS gene and its product in pathogenic strains of E. coli.

L2 ANSWER 4 OF 9 MEDLINE on STN DUPLICATE 2

2003025606. PubMed ID: 12531356. Functional mapping of protective epitopes within the rotavirus VP6 protein in mice belonging to different haplotypes. Choi Anthony H-C; McNeal Monica M; Basu Mitali; Bean Judy A; VanCott John L; Clements John D; Ward Richard L. (Division of Infectious Diseases, Cincinnati Children's Hospital Research Foundation, 3333 Burnet Avenue, Cincinnati, OH 45229-3039, USA.. anthony.choi@cchmc.org) . Vaccine, (2003 Jan 30) 21 (7-8) 761-7. Journal code: 8406899. ISSN: 0264-410X. Pub. country: Netherlands. Language: English.

AB We recently used "functional mapping" to locate protective epitopes in the carboxyl terminus (aa 197-397) of the VP6 protein (designated CD) of the EDIM strain of murine rotavirus [J. Virol. 74 (2000) 11574]. For this, H-2(d) BALB/c mice were given two intranasal (i.n.) immunizations (separated by 2 weeks) with VP6 or CD genetically-fused to maltose-binding protein, or with overlapping synthetic CD peptides, along with LT(R192G), a genetically-**attenuated E. coli** heat-labile toxin. The protective efficacies, i.e., percentage reductions in rotavirus shedding relative to control mice during 7 days following oral challenge with EDIM, were determined 4 weeks after the second immunization. Five of the 11 overlapping CD peptides stimulated significant protection (57-85%, P<0.05). Furthermore, chimeric VP6, the CD fragment, and a 14-amino-acid VP6 peptide within CD (RLSFQLMRPPNMTP), identified as a H-2(d)-restricted CD4 T cell epitope, were highly protective (93-98%, P<0.05). In this study, we continued to utilize functional mapping to show that the 14-mer peptide elicited significant protection (97.0%, P<0.05) in another H-2(d) mouse strain (DBA/2) but partial protection in H-2(b) 129 (39.2%) and C57Bl/6 (53.6%) as well as H-2(k) C3H (44.6%) mice. The first 13 amino acids of this 14-mer were necessary to induce maximal protection in H-2(d) mice. In addition, the H-2(b) 129 mice were immunized intranasally (i.n.) with 10 of the

synthetic CD peptides and 5 were found to induce significant protection (90-97%, P<0.05). We also performed functional mapping to identify MHC class I epitopes in rotavirus proteins. A class I-binding epitope for H-2(b) C57Bl/6 mice had previously been mapped by ex vivo CTL assays within the VP6 protein and two additional class I epitopes were identified by computer-based prediction. When examined for their protective efficacies by functional mapping, two of the three were found to be partially but not significantly protective (44 and 46%, P>0.05). To better determine the usefulness of our in vivo methods to identify MHC class I-binding epitopes, four epitopes from the outer capsid VP7 rotavirus protein determined in ex vivo assays were evaluated for their protective efficacies and two were found to be partially protective. Together, these studies show that functional mapping is useful in locating epitopes that are relevant to the development of subunit rotavirus vaccines.

L2 ANSWER 5 OF 9 MEDLINE on STN DUPLICATE 3

2002175184. PubMed ID: 11906760. The level of protection against rotavirus shedding in mice following immunization with a chimeric VP6 protein is dependent on the route and the coadministered adjuvant. Choi Anthony H; McNeal Monica M; Flint Jason A; Basu Mitali; Lycke Nils Y; Clements John D; Bean Judy A; Davis Heather L; McCluskie Michael J; VanCott John L; Ward Richard L. (Division of Infectious Diseases, Children's Hospital Medical Center, The Children's Hospital Research Foundation, 3333 Burnet Avenue, Cincinnati, OH 45229-3039, USA.. anthony.choi@chmcc.org) . Vaccine, (2002 Mar 15) 20 (13-14) 1733-40. Journal code: 8406899. ISSN: 0264-410X. Pub. country: England: United Kingdom. Language: English.

AB Intranasal (i.n.) immunization of BALB/c mice with chimeric murine rotavirus EDIM (epizootic diarrhea of infant mice) VP6 and **attenuated E. coli** heat-labile toxin (LT), LT(R192G), stimulated >99% protection against rotavirus shedding after EDIM challenge. Here, we evaluated other potential adjuvants with chimeric VP6 administered by two mucosal routes: i.n. and oral. Besides LT(R192G), the adjuvants examined included Adjumer, CpG oligodeoxynucleotides (CpG ODN), chimeric A1 subunit of cholera toxin (CTA1)-DD, and QS-21. All except QS-21 significantly (P<0.05) increased VP6-specific serum IgG responses after i.n. immunization, but none significantly increased these responses when administered orally. The i.n. delivery of chimeric VP6 alone induced both rotavirus IgG1 and IgG2a whose relative titers suggested a skewed Th2-like response. Inclusion of Adjumer greatly increased Th2-like responses, while CpG ODN shifted the response to a less Th2-like response. The adjuvants CTA1-DD, LT(R192G), QS-21 had no significant effect on ratios of IgG1/IgG2a titers. Following EDIM challenge of mice immunized i.n. with chimeric VP6 and either LT(R192G), CTA1-DD, Adjumer or CpG ODN, shedding was reduced >99, 95, 80, 74, respectively, relative to that found in unimmunized mice (P<0.05). QS-21 induced less protection (43%, not significant (N.S.)) while immunization with chimeric VP6 alone reduced shedding by only 16% (N.S.). Oral immunization with chimeric VP6 and all selected adjuvants except QS-21 was less effective than after i.n. immunization, with protection levels of 94 (P<0.05), 71 (P<0.05), 55, 35 and 28% for LT(R192G), QS-21, CpG ODN, CTA1-DD, and Adjumer, respectively, while immunization with chimeric VP6 alone gave no protection. Thus, different adjuvants induced different degrees of protection and oral immunization was generally less effective then the i.n. route.

L2 ANSWER 6 OF 9 EMBASE COPYRIGHT 2005 ELSEVIER INC. ALL RIGHTS RESERVED. on STN DUPLICATE 4

2001213213 EMBASE Differentiated attaching and effacing activities of porcine F4(+) enterotoxigenic and nonenterotoxigenic Escherichia coli strains. Vijtiuk N.; Bilic V.; Harapin I.; Vrbanac I.; Valpotic I.. I. Valpotic, Department of Biology, Veterinary Faculty, University of Zagreb, Heinzelova 55, 10000 Zagreb, Croatia. Periodicum Biologorum Vol. 103, No. 1, pp. 21-27 2001.
Refs: 36.

ISSN: 0031-5362. CODEN: PDBIAD
Pub. Country: Croatia. Language: English. Summary Language: English.
ED Entered STN: 20010628
AB Background and Purpose: In contrast to the development of vaccines against neonatal colibacillosis caused by porcine enterotoxigenic Escherichia coli (ETEC), comparatively little progress has been made in the development of vaccines against postweaning infections in swine. The vaccine candidate **attenuated E. coli** strains lacking plasmid encoding the heat-labile (LT) and heat-stable(STa and STb) enterotoxins were used as a live whole cell bacterins. The current study was undertaken to determine the differences, if any, in colonization of the small intestine, attaching-and-effacing lesion formation at the surface of villous epithelium, and ultrastructural characteristics of the enterocyte brush border microvilli between F4(+) ETEC strain and non-ETEC strains containing authentic or recombinant plasmid encoding for F4 fimbrial antigen using an experimental model of postweaning diarrheal (PWD) disease in swine. Differentiation between colonization patterns and histopathological appearance of the small intestine of pigs immunized with F4ac(+) either ETEC strain M1823 or recombinant/authentic non-ETEC strains 2407/1466, was studied in order to determine whether (or not) the fimbrial/toxin antigens produce morphologic changes in the intestinal mucosa. We also monitored adverse effects of oral immunization with these strains on health (incidence and severity of diarrhea) and performance (weight gain) of weaned pigs in this model. Materials and Methods: Five 4-week-old pigs per group (from a herd that was previously found to be highly susceptible to adhesion by F4(+) E. coli), were intragastrically inoculated (via orogastric tube) with either F4ac(+) (1466 or 2407) or F4(-) (1467) non-ETEC strains. Pigs in a fourth group were inoculated with F4ac(+) ETEC strain M1823 and the remaining 5 pigs that received broth containing 1.2% sodium bicarbonate were kept as noninoculated controls. The pigs were examined daily for 12 days. In this model, three conditions were observed in F4-susceptibte pigs: (1) acute fatal diarrheal disease; (2) moderate diarrhea and weight loss, and (3) no diarrhea or weight loss. The incidence of diarrhea, weight gain/loss, fecal shedding of the inoculum strain, and uttrastructural alterations in the villous epithelium of the small intestine were recorded. Results and Conclusions: Pigs inoculated with strain 2407 had much lower incidence of diarrhea and the highest weight gain. Diarrhea score and number of bacteria in 2407-treated pigs were significantly lower ($P < 0.05$ or $P < 0.01$, respectively) than those in M1823-treated pigs. Control and 2407-inoculated pigs gained weight substantially faster (25% and 30%, respectively) than 1466- (7%) or M1823-inoculated pigs did (10%). Ultrastructural examination of ileum from 1466- and M1823-inoculated pigs revealed moderate to severe lesions of microvilli of epithelial cells, respectively. There were no substantial differences in the brush border surface of ileal enterocytes between control pigs (with intact microvilli) and 1467- or 2407-inoculated pigs. However, the microvilli from 2407-inoculated pigs exhibited a minor structural irregularity caused by adhering E. coli cells. Conversely, the brush border membranes of M1823-inoculated pigs were heavily damaged and the epthelial villi were extensively disrupted. The results of this study indicate that different protective antigens delivered orally to pigs through vaccine candidate non-ETEC strains 1466 and 2407 can induce distinct colonization and attaching-effacing patterns in the small intestine, and may therefore produce various grades of lesions on the microvillous membranes of epithelial cells. Non-ETEC strain 2407 expressing F4ac(+) antigen induced only a mild microvillous damage, compared to the findings in pigs that were infected with a virulent F4ac(+) ETEC strain M1823. Although the recombinant non-ETEC strain 2407 was established in the present study as harmless bacterin suitable for development of live oral vaccine against PWD, further work is necessary to confirm its immunogenicity and define its mechanisms of action.

L2 ANSWER 7 OF 9 MEDLINE on STN DUPLICATE 5
1999370201. PubMed ID: 10438847. Antibody-independent protection against

rotavirus infection of mice stimulated by intranasal immunization with chimeric VP4 or VP6 protein. Choi A H; Basu M; McNeal M M; Clements J D; Ward R L. (Division of Infectious Diseases, Children's Hospital Medical Center, Cincinnati, Ohio 45229, USA.. achoi@chmcc.org) . Journal of virology, (1999 Sep) 73 (9) 7574-81. Journal code: 0113724. ISSN: 0022-538X. Pub. country: United States. Language: English.

AB This study was to determine whether individual rotavirus capsid proteins could stimulate protection against rotavirus shedding in an adult mouse model. BALB/c mice were intranasally or intramuscularly administered purified Escherichia coli-expressed murine rotavirus strain EDIM VP4, VP6, or truncated VP7 (TrVP7) protein fused to the 42.7-kDa maltose-binding protein (MBP). One month after the last immunization, mice were challenged with EDIM and shedding of rotavirus antigen was measured. When three 9-microg doses of one of the three rotavirus proteins fused to MBP were administered intramuscularly with the saponin adjuvant QS-21, serum rotavirus immunoglobulin G (IgG) was induced by each protein. Following EDIM challenge, shedding was significantly (P = 0.02) reduced (i.e., 38%) in MBP::VP6-immunized mice only. Three 9-micrograms doses of chimeric MBP::VP6 or MBP::TrVP7 administered intranasally with **attenuated E. coli** heat-labile toxin LT(R192G) also induced serum rotavirus IgG, but MBP::VP4 immunization stimulated no detectable rotavirus antibody. No protection against EDIM shedding was observed in the MBP::TrVP7-immunized mice. However, shedding was reduced 93 to 100% following MBP::VP6 inoculation and 56% following MBP::VP4 immunization relative to that of controls (P = <0.001). Substitution of cholera toxin for LT(R192G) as the adjuvant, reduction of the number of doses to 1, and challenge of the mice 3 months after the last immunization did not reduce the level of protection stimulated by intranasal administration of MBP::VP6. When MBP::VP6 was administered intranasally to B-cell-deficient microMt mice that made no rotavirus antibody, shedding was still reduced to <1% of that of controls. These results show that mice can be protected against rotavirus shedding by intranasal administration of individual rotavirus proteins and that this protection can occur independently of rotavirus antibody.

L2 ANSWER 8 OF 9 MEDLINE on STN DUPLICATE 6

88266211. PubMed ID: 2898844. Progress towards a vaccine against enterotoxigenic Escherichia coli. Kaper J B; Levine M M. (Department of Medicine, University of Maryland School of Medicine, Baltimore 21201.) Vaccine, (1988 Apr) 6 (2) 197-9. Ref: 22. Journal code: 8406899. ISSN: 0264-410X. Pub. country: ENGLAND: United Kingdom. Language: English.

AB A variety of approaches are being investigated in the development of a vaccine against enterotoxigenic Escherichia coli (ETEC). These approaches include purified fimbriae vaccines, toxoid vaccines, live **attenuated E. coli** vaccine strains and ETEC antigens expressed in carrier organisms. Studies of the pathogenesis and immune response to ETEC indicate that development of a vaccine against human ETEC is a realistic goal but considerable work remains before this goal is realized.

L2 ANSWER 9 OF 9 MEDLINE on STN DUPLICATE 7

85152406. PubMed ID: 3884174. Efficacy of S-2441, a synthetic oligopeptide, in a rat model for gram-negative bacteremia. Balis J U; Paterson J F; Fareed J; Claeson G; Desai U; Shelley S A. Circulatory shock, (1985) 15 (1) 5-14. Journal code: 0414112. ISSN: 0092-6213. Pub. country: United States. Language: English.

AB In vitro effects of S-2441, H-D-Pro-Phe-Arg-NH-Heptyl, include potent anti-bradykinin activity and broad-spectrum inhibition of serine proteases involved in the coagulation cascade. In this study, rats infused with 7.8 X 10(8) viable Escherichia coli were treated either with saline (group A) or with intravenous (0.1 mg) and intraperitoneal (0.4 mg) doses of S-2441 (group B). Survival rates for groups A and B were 68% and 98%, at 12 hours (P less than 0.001), and 37% and 73% at 24 hours (P less than 0.001), respectively. Hematologic studies revealed that S-2441 significantly inhibited E. coli-induced prolongation of prothrombin time

and partial thromboplastin time as well as a rapid decrease in the values of factor X, anti-thrombin III, and fibrinogen. In addition, S-2441 **attenuated E. coli**-induced hypoglycemia and a marked reduction of serum complement level. Ultrastructural evaluation of the liver demonstrated that S-2441 prevented the development of extensive sinusosoidal microthrombosis and hepatocellular necrosis. The results indicate that S-2441 affords protection in lethal gram-negative bacteremia owing in part to attenuation of disseminated intravascular coagulation and complement-mediated reactions. The findings are consistent with the concept that S-2441 and related oligopeptides modulate serine protease-mediated responses involving inhibition of active enzymes with competitive antagonism of pharmcologically active products formed during the activation of coagulation, fibrinolytic, kallikrein, and complement systems.

```
=> s heat killed E coli
L3          204 HEAT KILLED E COLI

=> s l3 and vaccine
L4           16 L3 AND VACCINE

=> dup remove l4
PROCESSING COMPLETED FOR L4
L5           10 DUP REMOVE L4 (6 DUPLICATES REMOVED)

=> d l5 1-10 cbib abs
```

L5 ANSWER 1 OF 10 MEDLINE on STN

2004340100. PubMed ID: 15189567. NF-kappaB p50 facilitates neutrophil accumulation during LPS-induced pulmonary inflammation. Mizgerd Joseph P; Lupa Michal M; Spieker Matt S. (Physiology Program, Harvard School of Public Health, Boston, MA, 02115 USA.. jmizgerd@hsph.harvard.edu) . BMC immunology [electronic resource], (2004 Jun 9) 5 (1) 10. Electronic Publication: 2004-06-09. Journal code: 100966980. ISSN: 1471-2172. Pub. country: England: United Kingdom. Language: English.

AB BACKGROUND: Transcription factors have distinct functions in regulating immune responses. During Escherichia coli pneumonia, deficiency of NF-kappaB p50 increases gene expression and neutrophil recruitment, suggesting that p50 normally limits these innate immune responses. p50-deficient mice were used to determine how p50 regulates responses to a simpler, non-viable bacterial stimulus in the lungs, E. coli lipopolysaccharide (LPS). RESULTS: In contrast to previous results with living E. coli, neutrophil accumulation elicited by E. coli LPS in the lungs was decreased by p50 deficiency, to approximately 30% of wild type levels. **Heat-killed E. coli** induced neutrophil accumulation which was not decreased by p50 deficiency, demonstrating that bacterial growth and metabolism were not responsible for the different responses to bacteria and LPS. p50 deficiency increased the LPS-induced expression of kappaB-regulated genes essential to neutrophil recruitment, including KC, MIP-2, ICAM-1, and TNF-alpha suggesting that p50 normally limited this gene expression and that decreased neutrophil recruitment did not result from insufficient expression of these genes. Neutrophils were responsive to the chemokine KC in the peripheral blood of p50-deficient mice with or without LPS-induced pulmonary inflammation. Interleukin-6 (IL-6), previously demonstrated to decrease LPS-induced neutrophil recruitment in the lungs, was increased by p50 deficiency, but LPS-induced neutrophil recruitment was decreased by p50 deficiency even in IL-6 deficient mice. CONCLUSION: p50 makes essential contributions to neutrophil accumulation elicited by LPS in the lungs. This p50-dependent pathway for neutrophil accumulation can be overcome by bacterial products other than LPS and does not require IL-6.

L5 ANSWER 2 OF 10 MEDLINE on STN DUPLICATE 1

96101747. PubMed ID: 7494229. Induction of T-cell immunity against Ras oncoproteins by soluble protein or Ras-expressing Escherichia coli. Fenton R G; Keller C J; Hanna N; Taub D D. (Division of Clinical Sciences, National Cancer Institute, National Cancer Institute-Frederick Cancer Research and Development Center (NCI-FCRDC), MD 21702, USA.) Journal of the National Cancer Institute, (1995 Dec 20) 87 (24) 1853-61. Journal code: 7503089. ISSN: 0027-8874. Pub. country: United States. Language: English.

AB BACKGROUND: Point mutations in the ras proto-oncogene that activate its oncogenic potential occur in approximately 30% of human cancers. Previous studies have demonstrated that T-cell immunity against some forms of mutant Ras proteins could be elicited, and some effectiveness against tumors expressing activated Ras has been reported. PURPOSE: The goal of this study was to determine if immunization of mice with two forms of mutant Ras protein can induce high levels of Ras mutation-specific T-cell immunity in vitro and tumor regression in vivo. METHODS: Mice (BALB/c or C3H/HeJ) were immunized subcutaneously at 2-week intervals with purified Ras oncoproteins mixed with the immunologic adjuvants Antigen Formulation or QS-21, both of which have been shown to enhance the induction of T-cell-mediated immunity when included as components of soluble protein **vaccines.** In some experiments, mice were immunized directly with heat-killed Escherichia coli that had been induced to express one of the mutant Ras proteins. Spleen cells plus lymph node cells from Ras-immunized mice were tested in vitro for lysis of syngeneic Ras-expressing tumor cells and proliferation in response to mutant Ras peptides. For some of the cytolytic activity experiments, the spleen cells were grown under TH1 conditions (growth in presence of interleukin 2, interferon gamma, and an antibody directed against interleukin 4 to stimulate a cell-mediated immune response) or TH2 conditions (growth in presence of interleukins 2 and 4 to stimulate a humoral immune response). The specificity of immunity was examined in vivo by challenge of Ras-immunized mice with syngeneic tumor cells expressing mutant Ras oncoproteins (HaBalb, i.e., BALB/c mouse cells expressing Ras with arginine substituted at amino acid position 12 [Arg 12 Ras]; C3HL61, i.e., C3H/HeJ mouse cells expressing Ras with leucine substituted at position 61 [Leu 61 Ras]). Ten mice per group were used in each experiment. RESULTS: Proliferative and cytolytic T-cell responses directed against the Arg 12 Ras protein were generated in BALB/c mice, resulting in protection against challenge with cells expressing Arg 12 Ras and therapeutic benefit in mice bearing established tumors expressing this protein. In C3H/HeJ mice, high levels of cytolytic and proliferative responses were induced against Leu 61 Ras. Immunization with **heat-killed E. coli** genetically engineered to express Leu 61 Ras also led to the induction of anti-Ras T-cell immunity. T cells grown under TH1 conditions were cytolytic against Ras-transformed tumor cells, whereas those grown under TH2 conditions were not. CONCLUSIONS: Immunization as described here leads to Ras mutation-specific antitumor immunity in vitro and in vivo, with therapeutic efficacy in an established tumor model.

L5 ANSWER 3 OF 10 SCISEARCH COPYRIGHT (c) 2005 The Thomson Corporation on STN

93:580095 The Genuine Article (R) Number: LX899. TOLERANCE AND IMMUNOGENICITY OF AN ORAL DOSE OF CVD 103-HGR, A LIVE ATTENUATED VIBRIO-CHOLERAE 01 STRAIN - A DOUBLE-BLIND-STUDY IN CHILEAN ADULTS. LAGOS R (Reprint); AVENDANO A; HORWITZ I; PRADO V; FERRECCIO C; SOTOMAYOR V; LOSONSKY G; WASSERMAN S S; CRYZ S; KAPER J B; LEVINE M M. HOSP ROBERTO DEL RIO, SERV SALUD METROPOLITANO NORTE, SANTIAGO, CHILE (Reprint); UNIV CHILE, FAC MED, DEPT PEDIAT, SANTIAGO, CHILE; UNIV CHILE, UNIDAD MICROBIOL, SANTIAGO, CHILE; UNIV MARYLAND, SCH MED, CTR VACCINE DEV, BALTIMORE, MD, 21201; INST SWISS SERUMS & VACCINES, BERN, SWITZERLAND. REVISTA MEDICA DE CHILE (AUG 1993) Vol. 121, No. 8, pp. 857-863. ISSN: 0034-9887. Pub. country: CHILE; USA; SWITZERLAND. Language: Spanish.

ABSTRACT IS AVAILABLE IN THE ALL AND IALL FORMATS

AB CVD 103-HgR is an attenuated, AB+, live, recombinant **vaccine** strain, developed by deletion of the toxA gen in a virulent Vibrio

cholerae 01, Inada classical strain (569B). In phase II studies conducted to date, CVD 103-HgR has been well tolerated and immunogenic in volunteers from both industrialized countries and cholera-endemic areas. In this study of safety, immunogenicity and excretion, 81 Chilean adults were randomly allocated to receive, in a double blind fashion, a single oral dose of 5x10(9) FU of CVD 103-HgR or placebo, (5x10(9) **heat-killed E. Coli** K12 organisms), in 100 ml of buffered water Side effects were assessed by daily visits to the participants. Immunogenicity, (vibriocidal seroconversion), was investigated in blood drawn before and on days 8 and 28 after immunization, while stool cultures to assess excretion of the **vaccine** strain were performed on specimens obtained on days 1 and 7. None of the participants, (40 vaccinees and 41 placebo recipients), experienced untoward effects during 30 minutes of close surveillance after ingestion of the preparation; upon follow up, neither adverse events were more frecuently reported by the vaccinees. 34/40 vaccinees, and 2/41 participants receiveing placebo had a significant raise, (> = fourfold), in their vibriocidal titers; (85 vs 2%, p < 0.001). The peak postimmunization geometric mean titer, (222), was ten fold higher than the baseline vibriocidal titer. The **vaccine** strain was recovered in stool cultures from 8 participants, one of them excreted the strain in both specimens. We conclude that CVD-103-HgR is safe and immunogenic in Chilean adults.

L5 ANSWER 4 OF 10 BIOSIS COPYRIGHT (c) 2005 The Thomson Corporation on STN
1985:233270 Document No.: PREV198579013266; BA79:13266. IMMUNOCONGLUTININ LEVELS IN CHICKS VACCINATED WITH SALMONELLA-GALLINARUM 9R SALMONELLA-PULLORUM E-79 OR ESCHERICHIA-COLI 020 **VACCINES** AND EXPERIMENTALLY INFECTED WITH SALMONELLA-GALLINARUM. JAISWAL T N [Reprint author]; MITTAL K R. COLLEGE OF VET SCIENCE AND ANIMAL HUSBANDRY, GUJARAT AGRIC UNIVERSITY, SK NAGAR, DANTIWADA, BANASKANTHA-395 506. Indian Veterinary Medical Journal, (1984) Vol. 8, No. 1, pp. 9-13. CODEN: IVMJDL. ISSN: 0250-5266. Language: ENGLISH.

AB Vaccination of chicks with live S. gallinarum (9R) **vaccines** with or without adjuvant caused an initial fall in the levels of pre-existing autostimulated immunoconglutinin (IK) by the 10th day but a slow increase in the IK level by 21st day postvaccination. Heat-killed S. pollorum (E79) and **heat-killed E. coli** (020) **vaccines** caused no such reduction in the IK level during the post-vaccination period. An increase in the IK level during post-vaccination period in these groups of chickens were observed. Challenge infection with S. gallinarium (V) in all the vaccinated groups of birds showed a marked decrease in IK level during the early challenge period indicating the involvement of IK in the host parasite reaction. The IK level increased by the 21st day post-challenge. Evidently, involvement of IK may help in host defense only in initial stages but eventually fail to protect chicks against S. gallinarum infection when the causative agent manages to enter the cells when both specific antibodies and nonspecific serum factor like IK fail to be effective.

L5 ANSWER 5 OF 10 MEDLINE on STN DUPLICATE 2
82190380. PubMed ID: 7042755. Induction of immunity against lethal Haemophilus influenzae type b infection by Escherichia coli core lipopolysaccharide. Marks M I; Ziegler E J; Douglas H; Corbeil L B; Braude A I. Journal of clinical investigation, (1982 Apr) 69 (4) 742-9. Journal code: 7802877. ISSN: 0021-9738. Pub. country: United States. Language: English.

AB Efforts to prevent Haemophilus influenzae type b (HIB) infections in infancy have been hampered by the low immunogenicity of capsular polysaccharide **vaccines** in children younger than 18 mos. In searching for alternate immunogens, we have studied the protective potential of polysaccharide-poor, lipid-rich endotoxin (LPS) core in experimental HIB infections. Because all gram-negative bacteria have similar LPS core structures, we were able to use as **vaccine** the J5 mutant of Escherichia coli 0111, the LPS of which consists only of core

components, and thus to avoid problems in interpretation arising from **vaccine** contamination with non-LPS HIB immunogens. Mice were given graded inocula of HIB and developed lethal infection analogous to human HIB disease when virulence was enhanced with mucin and hemoglobin. After active immunization with **heat-killed E. coli** J5, 40/50 (80%) of infected mice survived, compared with 14/50 (28%) of saline-immunized controls (P less than 0.005). Passive immunization with rabbit antiserum against E. coli J5 prevented lethal HIB infection when administered 24 or 72 h before or 3 h after infection. This protection was abolished by adsorption of antiserum with purified J5 LPS, with survival reduced from 14/24 to 0/24 (P less than 0.005). Furthermore, rabbit antiserum to purified J5 LPS gave just as potent protection against death as antiserum to whole J5 cells. These studies demonstrate that immunity to core LPS confers protection against experimental murine HIB infection and provide the framework for a new approach to prevention of human disease from HIB.

L5 ANSWER 6 OF 10 MEDLINE on STN

81281536. PubMed ID: 7023456. Consequences of active or passive immunization of turkeys against Escherichia coli O78. Arp L H. Avian diseases, (1980 Oct-Dec) 24 (4) 808-15. Journal code: 0370617. ISSN: 0005-2086. Pub. country: United States. Language: English.

AB Turkeys were injected at 7 and 14 days of age with live, heat-killed or formalin-killed Escherichia coli O78. Other turkeys were passively immunized at 22 days of age with hyperimmune serum produced against live or **heat-killed E. coli** O78. All turkeys were challenged at 24 days of age with E. coli O78. Turkeys immunized intramuscularly or intratracheally with live E. coli O78 were protected from death, whereas few turkeys given killed E. coli O78 were protected. Passively immunized turkeys were protected from death regardless of whether live or **heat-killed E. coli** O78 was used to produce the hyperimmune serum. Most turkeys that survived challenge developed septic polysynovitis 2--4 days after challenge.

L5 ANSWER 7 OF 10 MEDLINE on STN

76189304. PubMed ID: 818014. Antiviral activity of Brucella abortus preparations; separation of active components. Feingold D S; Keleti G; Youngner J S. Infection and immunity, (1976 Mar) 13 (3) 763-7. Journal code: 0246127. ISSN: 0019-9567. Pub. country: United States. Language: English.

AB Injection into mice of heat-killed Brucella abortus or aqueous ether-extracted B. abortus (Bru-pel) induced a "virus-type" interferon response, with peak titers at 6.5 h. The animals also were protected against challenge with otherwise lethal doses of Semliki forest virus. Extraction of either heated B. abortus or BRU-PEL with a mixture of chloroform-methanol (2:1, vol/vol) (C-M(yielded an insoluble residue (extracted cells) and a C-M extract. Neither extracted cells nor C-M extract alone induced interferon or afforded protection against Semliki forest virus infection in mice. Full interferon-inducing and protective activity was restored when extracted cells were recombined with C-M extract. C-M extract from heat-killed Escherichia coli also was effective in restoring activity to extracted Brucella cells. Neither **heat-killed E. coli** nor its C-M extract was active, nor was C-M estracted E. coli recombined with the C-M extract from B. abortus. These results suggest that the interferon-inducing and antiviral protective properties of B. abortus are constituted of a C-M-extractable component that is common to B. abortus and E. coli and an unextractable component that is unique to B. abortus.

L5 ANSWER 8 OF 10 CAPLUS COPYRIGHT 2005 ACS on STN

1974:567727 Document No. 81:167727 Intestinal antibody secretion in the young pig in response to oral immunization with Escherichia coli. Porter, P.; Kenworthy, R.; Noakes, D. E.; Allen, W. D. (Unilever Res., Sharnbrook/Bedford, UK). Immunology, 27(5), 841-53 (English) 1974.

CODEN: IMMUAM. ISSN: 0019-2805.

AB Intestinal immunoglobulins and antibodies in the local immune response to E. coli O somatic antigens was studied in young fistulated pigs. Antibody levels in intestinal secretion were raised for .apprx.2-3 weeks following a single local antigenic challenge with a heat-killed aqueous suspension of E. coli. A 2nd challenge provoked a similar response suggesting a lack of immunol. memory. Antibody activity in the secretions was predominantly associated with IgA and immunofluorescent studies of biopsy specimens from these pigs indicated that intestinal synthesis and secretion of IgA had begun by the 10th day of life. Studies of piglets reared with the sow indicated that oral immunization with E. coli antigen after 10 days of age stimulated intestinal antibody secretion before weaning at 3 weeks. The response of gnotobiotic pigs to oral immunization and infection was evaluated by immunofluorescent histol. of the intestinal mucosa. Repeated oral administration of **heat-killed E. coli** O8 gave an immunocyte response in the lamina propria numerically comparable with that produced by infection. The early response was dominated by cells of the IgM class whereas after 3 weeks IgA cells predominated. In the germ-free animal very few immunoglobulin-containing cells were detected. In vitro studies of antibacterial activity indicated that the most probable mechanism of immunol. control in the alimentary tract is bacteriostasis.

L5 ANSWER 9 OF 10 EMBASE on STN

75027648 EMBASE Document No.: 1975027648. The effect of active immunisation on ascending pyelonephritis in the rat. Radford N.J.; Chick S.; Ling R.; et al.. KRUF Inst. Ren. Dis., Welsh Nat. Sch. Med., Roy. Infirm., Cardiff, United Kingdom. J.PATH. Vol. 112, No. 3, pp. 169-175 1974.
CODEN: JPBAA7
Language: English.

AB In the rat, active immunization with **heat killed E. coli** serotype 078 **vaccine** produced a high titer of IgM anti O antibody after 14 days. At this time, lower titers of IgG anti O antibodies were found in some of the animals. These antibodies did not prevent bacterial invasion of the kidney nor did they affect the incidence or severity of the renal scarring following ascending infection with E. coli serotype 078. Fourteen days after immunization with a formalin killed **vaccine** very high titers of IgM and IgG anti K antibodies were noted; these were in excess of 1 in 5120. It was shown that these antibodies reduced the severity but not the frequency of renal scarring following ascending E. coli infection.

L5 ANSWER 10 OF 10 MEDLINE on STN

71078403. PubMed ID: 4923787. [Oral immunization against coli enteritis with streptomycin-dependent E. coli. V. Different efficiency of live Sm-d and **heat killed E. coli** O111 B4 **vaccine** in settling of the homologous Sm-r strain in mice with antibiotic sterilized intestine]. Untersuchungen zur oralen Immunisierung gegen Coli-Enteritis mit Streptomycin-dependenten Coli-Keimen. V. Unterschiedliche Wirksamkeit von Impfstoffen aus lebenden Streptomycin-dependenten und hitzeabgetoteten EC-O111 B4-Bakterien auf die Hemmung der Ansiedlung des homologen Streptomycin-resistenten Stammes bei darmsterilen Mausen. Lindek; Koch H. Zentralblatt fur Bakteriologie, Parasitenkunde, Infektionskrankheiten und Hygiene. 1. Abt. Medizinisch-hygienische Bakteriologie, Virusforschung und Parasitologie. Originale, (1970) 215 (3) 286-95. Journal code: 0337744. ISSN: 0372-8110. Pub. country: GERMANY, WEST: Germany, Federal Republic of. Language: German.

=> s E coli expressing Ara h
L6 0 E COLI EXPRESSING ARA H

=> s E coli

L7 351067 E COLI

=> s l7 and Ara h1
L8 0 L7 AND ARA H1

=> s l7 and peanut
L9 102 L7 AND PEANUT

=> s l9 and modified
L10 13 L9 AND MODIFIED

=> dup remove l10
PROCESSING COMPLETED FOR L10
L11 5 DUP REMOVE L10 (8 DUPLICATES REMOVED)

=> d l11 1-5 cbib abs

L11 ANSWER 1 OF 5 BIOSIS COPYRIGHT (c) 2005 The Thomson Corporation on STN
DUPLICATE 1
2005:80013 Document No.: PREV200500082205. Sensitization and allergic response and intervention therapy in animal models. Helm, Ricki M. [Reprint Author]; Burks, A. Wesley. ACHRIACNCDept Microbiol Immunol, Univ Arkansas Med Sci, ACHRI Slot 512-20b,1120 Marshall St, Little Rock, AR, 72202, USA. HelmRickiM@uams.edu. Journal of AOAC International, (November 2004) Vol. 87, No. 6, pp. 1441-1447. print.
ISSN: 1060-3271 (ISSN print). Language: English.
AB A review is presented of 3 murine models and a swine neonatal model used to investigate immunotherapeutic options. In Model 1, mutation of linear IgE-binding epitopeS of Ara h 1 for the preparation of a hypoallergenic Ara h 1 is discussed with respect to expression in transgenic tobacco plants and correct folding following expression in the pET16b construct. In Model 2, the mutations of Ara h 1 were assessed for use as an immunotherapeutic agent. Although some protective benefit was observed with the **modified** Ara h 1 protein, animals desensitized with heat-killed **E. coli** preparations showed increased protection to challenge. In Model 3, soybean homologs to **peanut** proteins were investigated to determine if soybean immunotherapy can potentially provide benefit to **peanut**-allergic subjects. Although some protection was provided, additional experimentation with respect to optimal doses for sensitization and challenge will need to be investigated. In Model 4, the neonatal swine model was used to profile different foods (low to moderate to high sensitizing) similar to food allergies in humans. Evidence suggests such feasiblity; however, threshold levels for sensitization and allergic responses will need additional study. In summary, murine and swine animal models are being used to address immunotherapeutic avenues and investigation into the mechanisms of food-allergic sensitization.

L11 ANSWER 2 OF 5 MEDLINE on STN DUPLICATE 2
2003475502. PubMed ID: 14550644. High-yield expression in Escherichia coli, purification, and characterization of properly folded major **peanut** allergen Ara h 2. Lehmann Katrin; Hoffmann Silke; Neudecker Philipp; Suhr Martin; Becker Wolf-Meinhard; Rosch Paul. (Lehrstuhl Biopolymere, Universitat Bayreuth 30, Universitaetsstrasse 30, 95440, Bayreuth, Germany.) Protein expression and purification, (2003 Oct) 31 (2) 250-9. Journal code: 9101496. ISSN: 1046-5928. Pub. country: United States. Language: English.
AB Allergic reactions to **peanuts** are a serious health problem because of their high prevalence, associated with potential severity, and chronicity. One of the three major allergens in **peanut**, Ara h 2, is a member of the conglutin family of seed storage proteins. Ara h 2 shows high sequence homology to proteins of the 2S albumin family. Presently, only very few structural data from allergenic proteins of this family exist. For a detailed understanding of the molecular mechanisms of food-induced allergies and for the development of therapeutic strategies

knowledge of the high-resolution three-dimensional structure of allergenic proteins is essential. We report a method for the efficient large-scale preparation of properly folded Ara h 2 for structural studies and report CD-spectroscopic data. In contrast to other allergenic 2S albumins, Ara h 2 exists as a single continuous polypeptide chain in **peanut** seeds, and thus heterologous expression in Escherichia coli was possible. Ara h 2 was expressed as Trx-His-tag fusion protein in **E. coli** Origami (DE3), a **modified E. coli** strain with oxidizing cytoplasm which allows the formation of disulfide bridges. It could be shown that recombinant Ara h 2, thus overexpressed and purified, and the allergen isolated from **peanuts** are identical as judged from immunoblotting, analytical HPLC, and circular dichroism spectra.

L11 ANSWER 3 OF 5 MEDLINE on STN DUPLICATE 3
2000417948. PubMed ID: 10910733. Use of **modified** BL21(DE3) Escherichia coli cells for high-level expression of recombinant **peanut** allergens affected by poor codon usage. Kleber-Janke T; Becker W M. (Department of Molecular and Biochemical Allergology, Research Center Borstel, Parkallee 22, Borstel, D-23845, Germany.. tamara.kleber_janke@gmx.de) . Protein expression and purification, (2000 Aug) 19 (3) 419-24. Journal code: 9101496. ISSN: 1046-5928. Pub. country: United States. Language: English.

AB We previously cloned a panel of **peanut** allergens by phage display technology. Examination of the codons used in these sequences indicated that most of the cDNAs contain an excess of the least used codons in Escherichia coli, namely AGG/AGA, that correspond to a minor tRNA, the product of the dnaY gene. To achieve high-level expression of the **peanut** allergens, the cDNAs were subcloned into an expression vector of the pET series (Novagen) in order to produce (His)(10)-tagged fusion proteins in conventional **E. coli** BL21(DE3) cells. The **peanut** allergens Ara h 1, Ara h 2, and Ara h 6 with an AGG/AGA codon content of 8-10% were only marginally expressed, whereas the **peanut** profilin Ara h 5, with an AGG/AGA codon content of only 0.8%, was efficiently expressed in these cells. Hence, by using **modified** BL21(DE3) **E. coli** cells, namely BL21-CodonPlus(DE3)-RIL cells (Stratagene) with extra copies of **E. coli** argU, ileY, and leuW tRNA genes, it was possible to attain high-level expression of the proteins affected by rare codon usage. IPTG-induced expression of several recombinant **peanut** allergens, such as Ara h 1, Ara h 2, and Ara h 6, was greatly increased in these special cells compared to the expression yield achieved by conventional **E. coli** hosts. The purification of the soluble and the insoluble fraction of Ara h 2 was performed by metal-affinity chromatography and yielded a total of about 30 mg (His)(10)-tagged recombinant protein per liter of culture of transformed BL21(DE3)CodonPlus-RIL cells. This is over 100 times more than achieved by production of Ara h 2 in conventional BL21(DE3) cells. Copyright 2000 Academic Press.

L11 ANSWER 4 OF 5 CAPLUS COPYRIGHT 2005 ACS on STN
2004:1039259 Document No. 142:3087 Process for preparing recombinant **peanut** agglutinin mutants for improved diagnosis of Thomsen-Friedenreich antigen (T-antigen) expression. Sharma, Vivek; Vijayan, Mamannamana; Surolia, Avadhesha (Indian Institute of Science, Molecular Biophysics Unit, India). Indian IN 182773 A 19990717, 29 pp. (English). CODEN: INXXAP. APPLICATION: IN 1996-MA461 19960322.

AB The invention relates to a process of preparing a **modified** protein, **peanut** agglutinin (PNA), with improved specificity for binding to specific ligands such as T-antigen comprising: synthesizing oligonucleotides for mutating PNA expressed in **E.coli** to improve its specificity for T-antigen, mutagenizing the PNA at an amino-acid residue which could interact only with its specific ligand, selecting the PNA mutants L212N and L212A based on the pattern of their restriction digestion for enzymes StyI and NaeI, confirming the PNA

mutants L212N and L212A by single stranded dideoxy DNA sequencing, expressing the PNA mutants L212N and L212A in **E.coli** cells to produce the **modified** protein PNA, solubilizing the **modified** protein PNA extracted from **E.coli** cells with guanidinium hydrochloride, and purifying the solubilized **modified** PNA using Ni2+ affinity chromatog.

L11 ANSWER 5 OF 5 CAPLUS COPYRIGHT 2005 ACS on STN
1992:52949 Document No. 116:52949 Cloning of plant desaturase cDNA and its expression in transgenic plants. Thompson, Gregory A.; Knauf, Vic C. (Calgene, Inc., USA). PCT Int. Appl. WO 9113972 A1 19910919, 130 pp. DESIGNATED STATES: W: CA, JP, US; RW: AT, BE, CH, DE, DK, ES, FR, GB, GR, IT, LU, NL, SE. (English). CODEN: PIXXD2. APPLICATION: WO 1991-US1746 19910314. PRIORITY: US 1990-494106 19900316; US 1990-567373 19900813; US 1990-615784 19901114.

AB CDNAs encoding plant fatty acid desaturases are cloned and expressed in bacterial and plant hosts. Altering the level of expression of the gene is useful in modulating the unsatd. fatty acid content of vegetable oils (no data). The safflower Δ9 fatty acyl CoA desaturase was purified and sequenced by standard methods, and from the sequence oligonucleotide probes and polymerase chain reaction primers were derived for screening a cDNA bank. The gene was transcribed in Escherichia coli but the enzyme was unable to use **E. coli** ferredoxin. A plant ferredoxin (e.g. from spinach) was necessary for enzymic activity. When the cDNA was placed under the control of a napin gene promoter and introduced into Brassica napus by Agrobacterium-mediated transformation regenerated plants showed some elevation of fatty acid desaturase activity.

=> s modified allergen
L12 438 MODIFIED ALLERGEN

=> s l12 and reduced IgE binding
L13 28 L12 AND REDUCED IGE BINDING

=> s l13 and expression
L14 1 L13 AND EXPRESSION

=> d l14 cbib abs

L14 ANSWER 1 OF 1 SCISEARCH COPYRIGHT (c) 2005 The Thomson Corporation on STN
2003:546621 The Genuine Article (R) Number: 693QZ. Analysis of the CD4(+) T cell responses to house dust mite allergoid. Kalinski P; Lebre M C; Kramer D; de Jong E C; van Schijndel J W P M; Kapsenberg M L (Reprint). Univ Amsterdam, Acad Med Ctr, Dept Cell Biol & Histol, POB 22700, NL-1100 DE Amsterdam, Netherlands (Reprint); Univ Amsterdam, Acad Med Ctr, Dept Cell Biol & Histol, NL-1100 DE Amsterdam, Netherlands; Univ Amsterdam, Acad Med Ctr, Dept Dermatol, NL-1105 AZ Amsterdam, Netherlands; Haarlems Allergenen Lab, Haarlem, Netherlands. ALLERGY (JUL 2003) Vol. 58, No. 7, pp. 648-656. Publisher: BLACKWELL MUNKSGAARD. 35 NORRE SOGADE, PO BOX 2148, DK-1016 COPENHAGEN, DENMARK. ISSN: 0105-4538. Pub. country: Netherlands. Language: English.
ABSTRACT IS AVAILABLE IN THE ALL AND IALL FORMATS

AB Background: **Modified allergen** extracts (allergoids) with **reduced IgE-binding** capacity are successfully used in immunotherapy of atopic allergy. Their reduced T-cell stimulatory capacity is less well studied and is a subject of the present study.

Methods: We compared the ability of native house dust mite extract (Dermatophagoides pteronyssinus ; HDM) and the glutaraldehyde-modified allergoid (HDM-GA) to induce the proliferation and cytokine production by fresh PBMC and by DC-stimulated polyclonal Th cells and HDM-specific Th cell clones.

Results: Freshly isolated T cells showed a partially reduced responsiveness to HDM-GA, differentially pronounced in different donors. HDM-specific Th cell clones prepared from three donors showed either a complete loss of reactivity to HDM-GA, or completely preserved responsiveness. The frequency of nonreactive clones was donor-dependent (2/3, 3/10 and 1/10). GA modification of HDM did not interfere with the cytokine production profile of HDM-specific T cell clones.

Conclusions: The reduced stimulatory potential of HDM-GA results mainly from a loss of certain Th cell epitopes, rather than impaired allergen uptake and presentation, or induction of suppressive factors. Varying frequencies of allergoid-nonreactive HDM-specific Th cells may result in differential responses of individual patients to immunotherapy.

=> dup remove l13
PROCESSING COMPLETED FOR L13
L15 10 DUP REMOVE L13 (18 DUPLICATES REMOVED)

=> d l15 1-10 cbib abs

L15 ANSWER 1 OF 10 MEDLINE on STN DUPLICATE 1
2004475201. PubMed ID: 15356555. Heat denaturation affects the ProDer p 1 IgE reactivity and downregulates the development of the specific allergic response. Magi Mauro; Garcia Lida; Vandenbranden Michel; Palmantier Remi; Jacquet Alain. (Service de Genetique Appliquee, Institut de Biologie et de Medecine Moleculaires, Universite Libre de Bruxelles, Gosselies, Belgium.) Journal of allergy and clinical immunology, (2004 Sep) 114 (3) 545-52. Journal code: 1275002. ISSN: 0091-6749. Pub. country: United States. Language: English.

AB BACKGROUND: **Modified allergens** with **reduced IgE-binding** activity represent an elegant approach to circumvent the risk of anaphylactic reactions in allergen-specific immunotherapy. OBJECTIVE: The current work investigated the effect of heat denaturation on the allergenic properties of recombinant ProDer p 1, a precursor form of the major house dust mite allergen Der p 1. METHODS: The IgE reactivity was estimated by direct and competition ELISA. The immunogenicity of heat-denatured ProDer p 1 was evaluated in naive and Der p 1-allergic mice. RESULTS: Heat denaturation in reducing conditions drastically reduced the in vitro ProDer p 1 IgE reactivity toward human allergic sera. In naive mice, heat-denatured ProDer p 1 generated mixed T(H)1-T(H)2 responses characterized by the absence of specific IgE with concomitant rise in specific IgG2a titers and the presence of IL-5 and IFN-gamma in splenocyte cultures. In contrast, natural Der p 1 or native ProDer p 1 induced typical strict T(H)2-biased allergic responses with strong IgG1 and IgE titers, whereas spleen cells exhibited only high IL-5 secretion. Moreover, native or heat-denatured ProDer p 1 vaccinations prevented airway eosinophil infiltrations after challenge. Although native or heat-treated ProDer p 1 adjuvanted with SBAS1b induced mixed T(H)1-T(H)2 responses in allergic mice, heat-denatured ProDer p 1, compared with native ProDer p 1, proved to be more effective in redirecting the T(H)2-allergic response toward T(H)1. CONCLUSION: Taken together, our results suggest that variants of Der p 1 with **reduced IgE-binding** reactivity could represent hypoallergenic molecules suitable for allergen-specific immunotherapy.

L15 ANSWER 2 OF 10 CAPLUS COPYRIGHT 2005 ACS on STN
2003:242373 Document No. 138:270285 Recombinant allergen with **reduced IgE binding** but undiminished T-cell antigenicity as immunotherapeutic of type I allergy. Deweerd, Nicole; Singh, Mohan Bir; Bhalla, Prem L.; Swoboda, Ines (The University of Melbourne, Australia). PCT Int. Appl. WO 2003025009 A1 20030327, 66 pp. DESIGNATED STATES: W: AE, AG, AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, BZ, CA, CH, CN, CO, CR, CU, CZ, DE, DK, DM, DZ, EC, EE, ES, FI, GB, GD, GE, GH, GM, HR, HU, ID, IL, IN, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MA, MD, MG, MK, MN, MW, MX, MZ, NO, NZ, OM, PH, PL, PT, RO,

RU, SD, SE, SG, SI, SK, SL, TJ, TM, TN, TR, TT, TZ, UA, UG, US, UZ, VC, VN, YU, ZA, ZM, ZW; RW: AT, BE, BF, BJ, CF, CG, CH, CI, CM, CY, DE, DK, ES, FI, FR, GA, GB, GR, IE, IT, LU, MC, ML, MR, NE, NL, PT, SE, SN, TD, TG, TR. (English). CODEN: PIXXD2. APPLICATION: WO 2002-AU1261 20020913. PRIORITY: AU 2001-7792 20010920.

AB The present invention relates generally to reagents useful in the immunotherapeutic or immunoprophylactic treatment of allergic diseases. More particularly, the present invention provides **modified allergens** exhibiting reduced IgE interactivity including reduced IgE production-stimulatory activity, while retaining T-cell antigenicity, which are useful in the immunomodulation of type I allergic disease conditions. The allergens comprise substitution, deletion or addition mutants or variants of Lol p 5, Phl p 5, Pao p 5 and immunol. related allergens. The present invention further contemplates a method of immunomodulation of allergic diseases such as type I allergic disease conditions by the administration of **modified allergens** exhibiting reduced IgE interactivity while retaining T-cell antigenicity.

L15 ANSWER 3 OF 10 CAPLUS COPYRIGHT 2005 ACS on STN

2003:855391 Document No. 139:363577 Modified anaphylactic food allergens with **reduced IgE-binding** ability for decreasing clinical reaction to allergy. Caplan, Michael J.; Sosin, Howard B.; Sampson, Hugh; Bannon, Gary A.; Burks, A. Wesley; Cockrell, Gael; Compadre, Cesar M.; Connaughton, Cathie; Helm, Ricki M.; King, Nina E.; Kopper, Randall A.; Maleki, Soheila J.; Rabjohn, Patrick A.; Shin, David S.; Stanley, J. Steven (USA). U.S. Pat. Appl. Publ. US 2003202980 A1 20031030, 194 pp., Cont.-in-part of U.S. Ser. No. 494,096. (English). CODEN: USXXCO. APPLICATION: US 2002-100303 20020318. PRIORITY: US 95-PV9455; 19951229; US 96-717933; 19960923; US 98-PV73283; 19980131; US 98-PV74633; 19980213; US 98-PV74624; 19980213; US 98-PV74590; 19980213; US 98-106872; 19980629; US 98-141220; 19980827; US 98-191593; 19981113; US 99-241101; 19990129; US 99-240557; 19990129; US 99-248674; 19990211; US 99-248673; 19990211; US 99-PV122560; 19990302; US 99-PV122565; 19990302; US 99-PV122566; 19990302; US 99-PV122450; 19990302; US 99-PV122452; 19990302; US 99-267719; 19990311; US 2000-494096; 20000128.

AB It has been determined that allergens, which are characterized by both humoral (IgE) and cellular (T-cell) binding sites, can be modified to be less allergenic by modifying the IgE binding sites. The IgE binding sites can be converted to non-IgE binding sites by altering as little as a single amino acid within the protein, preferably a hydrophobic residue towards the center of the IgE epitope, to eliminate IgE binding. Addnl. or alternatively a **modified allergen** with **reduced IgE binding** may be prepared by disrupting one or more of the disulfide bonds that are present in the natural allergen. The disulfide bonds may be disrupted chemical, e.g., by reduction and alkylation or by mutating one or more cysteine residues present in the primary amino acid sequence of the natural allergen. In certain embodiments, **modified allergens** are prepared by both altering one or more linear IgE epitopes and disrupting one or more disulfide bonds of the natural allergen. In certain embodiments, the methods of the present invention allow allergens to be modified while retaining the ability of the protein to activate T-cells, and, in some embodiments by not significantly altering or decreasing IgG binding capacity. The immunotherapeutics can be prepared in transgenic plants or animals; and administered in injection, aerosol, sublingual or topical form. The immunotherapeutics can also be encoded in gene for gene therapy and delivered by injecting into muscle or skin to induce tolerance. The Examples provided herein use peanut allergens to illustrate applications of the invention.

L15 ANSWER 4 OF 10 MEDLINE on STN DUPLICATE 2

2003295766. PubMed ID: 12823126. Analysis of the CD4+ T cell responses to house dust mite allergoid. Kalinski P; Lebre M C; Kramer D; De Jong E C; Van Schijndel J W P M; Kapsenberg M L. (Department of Cell Biology and Histology, Academic Medical Center, University of Amsterdam, Amsterdam,

The Netherlands.) Allergy, (2003 Jul) 58 (7) 648-56. Journal code: 7804028. ISSN: 0105-4538. Pub. country: Denmark. Language: English.

AB BACKGROUND: **Modified allergen** extracts (allergoids) with **reduced IgE-binding** capacity are successfully used in immunotherapy of atopic allergy. Their reduced T-cell stimulatory capacity is less well studied and is a subject of the present study. METHODS: We compared the ability of native house dust mite extract (Dermatophagoides pteronyssinus; HDM) and the glutaraldehyde-modified allergoid (HDM-GA) to induce the proliferation and cytokine production by fresh PBMC and by DC-stimulated polyclonal Th cells and HDM-specific Th cell clones. RESULTS: Freshly isolated T cells showed a partially reduced responsiveness to HDM-GA, differentially pronounced in different donors. HDM-specific Th cell clones prepared from three donors showed either a complete loss of reactivity to HDM-GA, or completely preserved responsiveness. The frequency of nonreactive clones was donor-dependent (2/3, 3/10 and 1/10). GA modification of HDM did not interfere with the cytokine production profile of HDM-specific T cell clones. CONCLUSIONS: The reduced stimulatory potential of HDM-GA results mainly from a loss of certain Th cell epitopes, rather than impaired allergen uptake and presentation, or induction of suppressive factors. Varying frequencies of allergoid-nonreactive HDM-specific Th cells may result in differential responses of individual patients to immunotherapy.

L15 ANSWER 5 OF 10 CAPLUS COPYRIGHT 2005 ACS on STN DUPLICATE 3

2003:906632 Correction of: 2002:736063 Document No. 139:349665 Correction of: 137:277814 Modified anaphylactic food allergens with **reduced IgE-binding** ability for decreasing clinical reaction to allergy. Caplan, Michael; Sosin, Howard; Sampson, Hugh; Bannon, Gary A.; Burks, Wesley A.; Cockrell, Gael; Compadre, Cesar M.; Connaughton, Cathie; Helm, Ricki M.; King, Nina E.; Kopper, Randall A.; Maleki, Sohelia J.; Rabjohn, Patrick A.; Shin, David S.; Stanley, J. Steven (Panacea Pharmaceuticals, USA; et al.). PCT Int. Appl. WO 2002074250 A2 20020926, 299 pp. DESIGNATED STATES: W: AE, AG, AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, BZ, CA, CH, CN, CO, CR, CU, CZ, DE, DK, DM, DZ, EC, EE, ES, FI, GB, GD, GE, GH, GM, HR, HU, ID, IL, IN, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MA, MD, MG, MK, MN, MW, MX, MZ, NO, NZ, OM, PH, PL, PT, RO, RU, SD, SE, SG, SI, SK, SL, TJ, TM, TN, TR, TT, TZ, UA, UG, US, UZ, VN, YU, ZA, ZM, ZW; RW: AT, BE, BF, BJ, CF, CG, CH, CI, CM, CY, DE, DK, ES, FI, FR, GA, GB, GR, IE, IT, LU, MC, ML, MR, NE, NL, PT, SE, SN, TD, TG, TR. (English). CODEN: PIXXD2. APPLICATION: WO 2002-US9108 20020318. PRIORITY: US 2001-PV276822 20010316.

AB It has been determined that allergens, which are characterized by both humoral (IgE) and cellular (T-cell) binding sites, can be modified to be less allergenic by modifying the IgE binding sites. The IgE binding sites can be converted to non-IgE binding sites by altering as little as a single amino acid within the protein, preferably a hydrophobic residue towards the center of the IgE epitope, to eliminate IgE binding. Addnl. or alternatively a **modified allergen** with **reduced IgE binding** may be prepared by disrupting one or more of the disulfide bonds that are present in the natural allergen. The disulfide bonds may be disrupted chemical, e.g., by reduction and alkylation or by mutating one or more cysteine residues present in the primary amino acid sequence of the natural allergen. In certain embodiments, **modified allergens** are prepared by both altering one or more linear IgE epitopes and disrupting one or more disulfide bonds of the natural allergen. In certain embodiments, the methods of the present invention allow allergens to be modified while retaining the ability of the protein to activate T-cells, and, in some embodiments by not significantly altering or decreasing IgG binding capacity. The Examples provided herein use peanut allergens to illustrate applications of the invention.

L15 ANSWER 6 OF 10 MEDLINE on STN DUPLICATE 4

2001262411. PubMed ID: 11306930. Engineering, characterization and in vitro efficacy of the major peanut allergens for use in immunotherapy.

Bannon G A; Cockrell G; Connaughton C; West C M; Helm R; Stanley J S; King N; Rabjohn P; Sampson H A; Burks A W. (Department of Biochemistry and Molecular Biology, Arkansas Children's Hospital Research Institute, Little Rock 72205, USA.. bannongarya@exchnage.uams.edu) . International archives of allergy and immunology, (2001 Jan-Mar) 124 (1-3) 70-2. Journal code: 9211652. ISSN: 1018-2438. Pub. country: Switzerland. Language: English.

AB BACKGROUND: Numerous strategies have been proposed for the treatment of peanut allergies, but despite the steady advancement in our understanding of atopic immune responses and the increasing number of deaths each year from peanut anaphylaxis, there is still no safe, effective, specific therapy for the peanut-sensitive individual. Immunotherapy would be safer and more effective if the allergens could be altered to reduce their ability to initiate an allergic reaction without altering their ability to desensitize the allergic patient. METHODS: The cDNA clones for three major peanut allergens, Ara h 1, Ara h 2, and Ara h 3, have been cloned and characterized. The IgE-binding epitopes of each of these allergens have been determined and amino acids critical to each epitope identified. Site-directed mutagenesis of the allergen cDNA clones, followed by recombinant production of the **modified allergen**, provided the reagents necessary to test our hypothesis that hypoallergenic proteins are effective immunotherapeutic reagents for treating peanut-sensitive patients. Modified peanut allergens were subjected to immunoblot analysis using peanut-positive patient sera IgE, T cell proliferation assays, and tested in a murine model of peanut anaphylaxis. RESULTS: In general, the **modified allergens** were poor competitors for binding of peanut-specific IgE when compared to their wild-type counterpart. The **modified allergens** demonstrated a greatly **reduced IgE-binding** capacity when individual patient serum IgE was compared to the binding capacity of the wild-type allergens. In addition, while there was considerable variability between patients, the **modified allergens** retained the ability to stimulate T cell proliferation. CONCLUSIONS: These **modified allergen** genes and proteins should provide a safe immunotherapeutic agent for the treatment of peanut allergy.

L15 ANSWER 7 OF 10 MEDLINE on STN DUPLICATE 5

2000429040. PubMed ID: 10925258. T cell reactivity with allergoids: influence of the type of APC. Kahlert H; Grage-Griebenow E; Stuwe H T; Cromwell O; Fiebig H. (Allergopharma Joachim Ganzer KG, Reinbek, Germany.. allergopharmakg@csi.com) . Journal of immunology (Baltimore, Md. : 1950), (2000 Aug 15) 165 (4) 1807-15. Journal code: 2985117R. ISSN: 0022-1767. Pub. country: United States. Language: English.

AB The use of allergoids for allergen-specific immunotherapy has been established for many years. The characteristic features of these chemically **modified allergens** are their strongly **reduced IgE binding** activity compared with the native form and the retained immunogenicity. T cell reactivity of chemically **modified allergens** is documented in animals, but in humans indirect evidence of reactivity has been concluded from the induction of allergen-specific IgG during immunotherapy. Direct evidence of T cell reactivity was obtained recently using isolated human T cells. To obtain further insight into the mechanism of action of allergoids, we compared the Ag-presenting capacity of different APC types, including DC and macrophages, generated from CD14+ precursor cells from the blood of grass pollen allergic subjects, autologous PBMC, and B cells. These APC were used in experiments together with Phl p 5-specific T cell clones under stimulation with grass pollen allergen extract, rPhl p 5b, and the respective allergoids. Using DC and macrophages, allergoids exhibited a pronounced and reproducible T cell-stimulating capacity. Responses were superior to those with PBMC, and isolated B cells failed to present allergoids. Considerable IL-12 production was observed only when using the DC for Ag presentation of both allergens and allergoids. The amount of IL-10 in supernatants was dependent on the phenotype of the

respective T cell clone. High IL-10 production was associated with suppressed IL-12 production from the DC in most cases. In conclusion, the reactivity of Th cells with allergoids is dependent on the type of the APC.

L15 ANSWER 8 OF 10 CAPLUS COPYRIGHT 2005 ACS on STN
1999:495393 Document No. 131:143513 Methods and reagents for decreasing allergic reactions. Sosin, Howard; Bannon, Gary A.; Burks, A. Wesley, Jr.; Sampson, Hugh A. (University of Arkansas, USA; Mt. Sinai School of Medicine, University of New York). PCT Int. Appl. WO 9938978 A1 19990805, 46 pp. DESIGNATED STATES: W: AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, CA, CH, CN, CU, CZ, DE, DK, EE, ES, FI, GB, GE, GH, GM, HR, HU, ID, IL, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MD, MG, MK, MN, MW, MX, NO, NZ, PL, PT, RO, RU, SD, SE, SG, SI, SK, SL, TJ, TM, TR, TT, UA, UG, UZ, VN, YU, ZW, AM, AZ, BY, KG, KZ, MD, RU, TJ, TM; RW: AT, BE, BF, BJ, CF, CG, CH, CI, CM, CY, DE, DK, ES, FI, FR, GA, GB, GR, IE, IT, LU, MC, ML, MR, NE, NL, PT, SE, SN, TD, TG. (English). CODEN: PIXXD2. APPLICATION: WO 1999-US2031 19990129. PRIORITY: US 1998-PV73283 19980131; US 1998-PV74590 19980213; US 1998-PV74624 19980213; US 1998-PV74633 19980213; US 1998-141220 19980827.

AB It has been determined that allergens, which are characterized by both humoral (IgE) and cellular (T cell) binding sites, can be modified to be less allergenic by modifying the IgE binding sites. The IgE binding sites can be converted to non-IgE binding sites by masking the site with a compound that prevents IgE binding or by altering as little as a single amino acid within the protein, most typically a hydrophobic residue towards the center of the IgE-binding epitope, to eliminate IgE binding. The method allows the protein to be altered as minimally as possible, other than within the IgE-binding sites, while retaining the ability of the protein to activate T cells, and, in some embodiments by not significantly altering or decreasing IgG binding capacity. The examples use peanut allergens to demonstrate alteration of IgE binding sites. The critical amino acids within each of the IgE binding epitopes of the peanut protein that are important to Ig binding have been determined Substitution of even a single amino acid within each of the epitopes led to loss of IgE binding. Although the epitopes shared no common amino acid sequence motif, the hydrophobic residues located in the center of the epitope appeared to be most critical to IgE binding.

L15 ANSWER 9 OF 10 MEDLINE on STN DUPLICATE 6
97414840. PubMed ID: 9269506. Preseasonal specific immunotherapy with modified Phleum pratense allergenic extracts: tolerability and effects. Ricca V; Ciprandi G; Pesce G; Riccio A; Varese P; Pecora S; Canonica G W. (Servizio di Allergologia, Ospedale Koelliker de Missionari di Maria S.S. Consolata, Torino, Italia.) Allergologia et immunopathologia, (1997 Jul-Aug) 25 (4) 167-75. Journal code: 0370073. ISSN: 0301-0546. Pub. country: Spain. Language: English.

AB The preparation of chemically **modified allergens**, with a **reduced IgE binding** capacity (responsible for side effects with traditional immunotherapy) but with the same or greater immunogenic activity, is one of the paths followed to obtain better results with specific immunotherapy (IT). The aim of the study was to evaluate the tolerability and effects of an extract Phleum pratense, modified with glutaraldehyde and absorbed on aluminium hydroxide, in controlling the seasonal symptomatology induced by grass pollen in a group of 10 monosensitized patients, compared to a group of 10 similar patients not treated with specific IT but with drugs alone. The monitoring parameters were: 1) Clinical: a) symptomatology after specific conjunctival provocation test (pre and post seasonal) and during the natural exposure to the allergen b) drug consumption. 2) Immunological (peripheral blood eosinophils, total and specific IgE, total specific IgG). 3) Cytological, before, during and after the pollen season. CONCLUSIONS: In subjects treated with specific IT a) both the overall symptomatology and the drug consumption resulted significantly reduced compared to the controls ($p = 0.045$); b) the phlogistic infiltrate showed

a tendency to decrease during the pollen season; c) the peripheral blood eosinophils, total and specific IgE and IgG did not show any significant variation compared to the controls; d) no systemic reactions occurred and there were only two slight local reactions.

L15 ANSWER 10 OF 10 MEDLINE on STN DUPLICATE 7
97163754. PubMed ID: 9010561. Preseasonal specific immunotherapy with modified phleum pratense allergenic extracts: tolerability and effects. Vittorio R; Giorgio C; Giampaola G; Annamaria R; Paola V; Silvia P; Walter C G. (Servizio di allergologia, ospedale koelliker dei missionari di Maria S.S. Consolata, Torino, Italia.) Allergologia et immunopathologia, (1996 Nov-Dec) 24 (6) 255-62. Journal code: 0370073. ISSN: 0301-0546. Pub. country: Spain. Language: English.

AB The preparation of chemically **modified allergens**, with a **reduced IgE binding** capacity (responsible for side effects with traditional immunotherapy) but with the same or greater immunogenic activity, is one of the paths followed to obtain better results with specific immunotherapy (IT). The aim of the study was to evaluate the tolerability and effects of extracts of Phleum pratense, modified with glutaraldehyde and absorbed on aluminium hydroxide, in controlling the seasonal symptomatology induced by grass pollen in a group of 10 monosensitized patients, compared to a group of 10 similar patients not treated with specific IT but with drugs alone. The monitoring parameters were: 1) Clinical: a) symptomatology after specific conjuctival provocation test (pre and post seasonal) and during the natural exposure to the allergen b) drug consumption. 2) Immunological (peripheral blood eosinophils, total and specific IgE, total specific IgG). 3) Cytological, before, during and after the pollen season. Conclusions: in subjects treated with specific IT a) both the overall symptomatology and the drug consumption resulted significantly reduced compared to the controls ($p = 0.045$); b) the phlogistic infiltrate showed a tendency to decrease during the pollen season; c) the peripheral blood eosinophils, total and specific IgE and IgG did not show any significant variation compared to the controls; d) no systemic reactions occurred and there were only two slight local reactions.

=> s modified peanut allergen
L16 10 MODIFIED PEANUT ALLERGEN

=> dup remove l16
PROCESSING COMPLETED FOR L16
L17 4 DUP REMOVE L16 (6 DUPLICATES REMOVED)

=> d l17 1-4 cbib abs

L17 ANSWER 1 OF 4 BIOSIS COPYRIGHT (c) 2005 The Thomson Corporation on STN
2003:347653 Document No.: PREV200300347653. Persistent effect of immunotherapy with "engineered" recombinant peanut protein and a bacterial adjuvant on peanut hypersensitivity. Li, X. [Reprint Author]; Srivastava, K. D. [Reprint Author]; Grishin, A. [Reprint Author]; Stanley, J. S.; Burks, A. W.; Sampson, H. A. [Reprint Author]. Pediatrics, Mount Sinai School of Medicine, New York, NY, USA. Journal of Allergy and Clinical Immunology, (February 2003) Vol. 111, No. 2 Abstract Supplement, pp. S195. print. Meeting Info.: AAAAI 60th Anniversary Meeting. Denver, CO, USA. March 07-12, 2003. American Academy of Allergy, Asthma and Immunology. CODEN: JACIBY. ISSN: 0091-6749. Language: English.

L17 ANSWER 2 OF 4 BIOSIS COPYRIGHT (c) 2005 The Thomson Corporation on STN DUPLICATE 1
2002:301808 Document No.: PREV200200301808. Immunotherapy with **modified peanut allergens** in a murine model of peanut allergy. Srivastava, Kamal D. [Reprint author]; Li, Xiu-Min [Reprint author]; King, Nina; Stanley, Steven; Bannon, Gary A.; Burks, Wesley; Sampson, Hugh A. [Reprint author]. Mount Sinai Medical Center, New

York, NY, USA. Journal of Allergy and Clinical Immunology, (January, 2002) Vol. 109, No. 1 Supplement, pp. S287. print.
Meeting Info.: 58th Annual Meeting of the American Academy of Allergy, Asthma and Immunology. New York, NY, USA. March 01-06, 2002. American Academy of Allergy, Asthma, and Immunology.
CODEN: JACIBY. ISSN: 0091-6749. Language: English.

L17 ANSWER 3 OF 4 MEDLINE on STN DUPLICATE 2
2002303208. PubMed ID: 12045419. Recent advances in peanut allergy. Hourihane Jonathan O'B. (Division of Infection Inflammation and Repair, Southampton University Hospitals NHS Trust, Southampton, UK.. j.hourihane@soton.ac.uk) . Current opinion in allergy and clinical immunology, (2002 Jun) 2 (3) 227-31. Ref: 44. Journal code: 100936359. ISSN: 1528-4050. Pub. country: United States. Language: English.
AB Peanut remains preeminent as the food allergen most associated with severe and fatal allergic reactions. Reactions are frequent despite patients' best efforts to avoid peanut. In the future, better information sharing and communication between families and both schools and restaurants may lead to a decrease in the rate of severe reactions induced by exposure to peanut outside the home. Reaction severity may increase over time but up to 25% of young peanut allergic individuals may outgrow their peanut allergy. Personalized care plans and education programmes may have an impact on avoidance of peanut and on the appropriate responses of caregivers. Peanut's allergenicity may be affected by the method of cooking, with roasted peanuts appearing more allergenic than boiled or fried peanuts. Immunotherapy with **modified peanut allergens** and DNA based vaccines may soon move from animal studies to clinical trials.

L17 ANSWER 4 OF 4 MEDLINE on STN DUPLICATE 3
2001262411. PubMed ID: 11306930. Engineering, characterization and in vitro efficacy of the major peanut allergens for use in immunotherapy. Bannon G A; Cockrell G; Connaughton C; West C M; Helm R; Stanley J S; King N; Rabjohn P; Sampson H A; Burks A W. (Department of Biochemistry and Molecular Biology, Arkansas Children's Hospital Research Institute, Little Rock 72205, USA.. bannongarya@exchnage.uams.edu) . International archives of allergy and immunology, (2001 Jan-Mar) 124 (1-3) 70-2. Journal code: 9211652. ISSN: 1018-2438. Pub. country: Switzerland. Language: English.
AB BACKGROUND: Numerous strategies have been proposed for the treatment of peanut allergies, but despite the steady advancement in our understanding of atopic immune responses and the increasing number of deaths each year from peanut anaphylaxis, there is still no safe, effective, specific therapy for the peanut-sensitive individual. Immunotherapy would be safer and more effective if the allergens could be altered to reduce their ability to initiate an allergic reaction without altering their ability to desensitize the allergic patient. METHODS: The cDNA clones for three major peanut allergens, Ara h 1, Ara h 2, and Ara h 3, have been cloned and characterized. The IgE-binding epitopes of each of these allergens have been determined and amino acids critical to each epitope identified. Site-directed mutagenesis of the allergen cDNA clones, followed by recombinant production of the modified allergen, provided the reagents necessary to test our hypothesis that hypoallergenic proteins are effective immunotherapeutic reagents for treating peanut-sensitive patients. **Modified peanut allergens** were subjected to immunoblot analysis using peanut-positive patient sera IgE, T cell proliferation assays, and tested in a murine model of peanut anaphylaxis. RESULTS: In general, the modified allergens were poor competitors for binding of peanut-specific IgE when compared to their wild-type counterpart. The modified allergens demonstrated a greatly reduced IgE-binding capacity when individual patient serum IgE was compared to the binding capacity of the wild-type allergens. In addition, while there was considerable variability between patients, the modified allergens retained the ability to stimulate T cell proliferation. CONCLUSIONS: These modified allergen genes and proteins should provide a safe immunotherapeutic agent for the treatment of peanut allergy.

=> s peanut allergen
L18 829 PEANUT ALLERGEN

=> s l18 and Ara h1
L19 33 L18 AND ARA H1

=> dup remove l19
PROCESSING COMPLETED FOR L19
L20 21 DUP REMOVE L19 (12 DUPLICATES REMOVED)

=> s l20 and modified
L21 1 L20 AND MODIFIED

=> d l21 cbib abs

L21 ANSWER 1 OF 1 BIOSIS COPYRIGHT (c) 2005 The Thomson Corporation on STN
2002:301398 Document No.: PREV200200301398. Immunotherapy for peanut allergy using **modified** allergens and a bacterial adjuvant. Stanley, Joseph Steve [Reprint author]; Buzen, Fred [Reprint author]; Cockrell, Gael [Reprint author]; West, Mike [Reprint author]; Srivastava, Kamal D.; Li, X. M.; Sampson, Hugh A.; Burks, Wesley [Reprint author]; Bannon, Gary A. [Reprint author]. University of Arkansas, Little Rock, AR, USA. Journal of Allergy and Clinical Immunology, (January, 2002) Vol. 109, No. 1 Supplement, pp. S93. print.
Meeting Info.: 58th Annual Meeting of the American Academy of Allergy, Asthma and Immunology. New York, NY, USA. March 01-06, 2002. American Academy of Allergy, Asthma, and Immunology.
CODEN: JACIBY. ISSN: 0091-6749. Language: English.

=> dup remove l20
PROCESSING COMPLETED FOR L20
L22 21 DUP REMOVE L20 (0 DUPLICATES REMOVED)

=> d l22 1-21 cbib abs

L22 ANSWER 1 OF 21 MEDLINE on STN .
2005064300. PubMed ID: 15692187. The cDNA cloning of conarachin gene and its expression in developing peanut seeds. Wang Lei; Yan Yong-Sheng; Liao Bin; Lin Xiao-Dong; Huang Shang-Zhi. (Department of Bioscience and Biotechnology, Zhongshan University, Guangzhou 510275, China.) Zhi Wu Sheng Li Yu Fen Zi Sheng Wu Xue Xue Bao, (2005 Feb) 31 (1) 107-10.
Journal code: 101156321. ISSN: 1671-3877. Pub. country: China. Language: Chinese.
AB In order to clone the genes of peanut seed storage proteins, a cDNA library of mid-maturation stage cotyledons of peanut (Arachis hypogaea L. Shanyou 523) was constructed. After immunoscreening with polyclonal antibody against conarachin from peanut seeds, two cDNA sequences named Ahy alpha and Ahy beta of conarachin genes were obtained. BLAST these sequences in GenBank showed that they share high homology with the genes of legume 7S globulins. One of the sequences (Ahy beta) was found to be identical to part of the published complete cDNA sequence of the **peanut allergen, Ara h1** p17. The
other fragment (Ahy alpha) was almost identical to parts of the published complete cDNA sequence of the **peanut allergen,**
Ara h1 p41b. Results of southern analysis show that
they belong to a small gene family (the result is not shown). These sequences were expressed only in developing peanut seeds. To isolate the genes involved in seed storage protein and to understand the gene expression during peanut seed development, the sequences of 414 ESTs were determined and further analyzed by BLAST searches and categorized functionally. Five rESTs (representative ones) of arahin, two rESTs of

conarachin and six rESTs of conglutin-like proteins were isolated. 17.0% of total transcripts isolated in this study are involved in peanut seed storage proteins.

L22 ANSWER 2 OF 21 CAPLUS COPYRIGHT 2005 ACS on STN
2004:539630 Document No. 141:224207 The major **peanut allergen** Ara h 1 and its cleaved-off N-terminal peptide; Possible implications for **peanut allergen** detection. Wichers, Harry J.; De Beijer, Thomas; Savelkoul, Huub F. J.; Van Amerongen, Aart (Agrotechnology and Food Innovations, Wageningen University and Research Center, Wageningen, 6708 PD, Neth.). Journal of Agricultural and Food Chemistry, 52(15), 4903-4907 (English) 2004. CODEN: JAFCAU. ISSN: 0021-8561. Publisher: American Chemical Society.

AB Ara h 1 was purified from raw peanuts (Arachis hypogaea L.) in the presence or absence of protease inhibitors. N-Terminal amino acid sequences were determined after western blotting. Both purification procedures proved to be very consistent and resulted in identical chromatog. and electrophoretic behavior of Ara h 1 and in the isolation of identical proteins of .apprx.64 kDa with RS/H_PPGERTRG as the N-terminal amino acid sequence. Consequently, purified Ara h 1 appears to be truncated at the N-terminal side. The observations strongly suggest that Ara h 1 occurs physiol. as a protein of which the first 84 and 78 amino acids, resp., are cleaved off in planta upon maturation of the protein. On the basis of epitope mapping, the cleaved-off N-terminal peptide contains three allergenic epitopes, of which two are major. These truncated epitopes will go undetected in assays when purified Ara h 1 from peanuts is used as reference material. Patients' sera, however, contain IgE-type antibodies against the epitopes that are contained in the cleaved-off peptide, implying that the peptide, or part of it, is still present in peanuts that are consumed. Possible consequences of this exposure to these three epitopes are discussed. On the basis of literature data the cleaved-off peptide is hypothesized to have antifungal activity.

L22 ANSWER 3 OF 21 CAPLUS COPYRIGHT 2005 ACS on STN
2004:385944 Document No. 141:224142 **Peanut allergen** (Ara h 1) detection in foods containing chocolate. Pomes, A.; Vinton, R.; Chapman, M. D. (Indoor Biotechnologies, Inc., Charlottesville, VA, 22903, USA). Journal of Food Protection, 67(4), 793-798 (English) 2004. CODEN: JFPRDR. ISSN: 0362-028X. Publisher: International Association for Food Protection.

AB The authors report the optimization of conditions for measuring a major **peanut allergen**, Ara h 1, in chocolate with the use of a two-site monoclonal antibody sandwich ELISA. Ara h 1 was extracted from peanut in the presence or absence of chocolate with phosphate buffer, salt, and three dried milks (goat, soy, or nonfat) (0 to 25% wt/vol) for 15 min at 60° or for 2.5 h at room temperature The best conditions for Ara h 1 extraction in the presence of chocolate were 5% nonfat dry milk for 2.5 h at room temperature Spiking expts. of chocolate with peanut confirmed improvement of the extraction: Ara h 1 was detected in extns. of 0.16 to 0.33% peanut in chocolate. Interestingly, the best conditions for Ara h 1 extraction were different for peanut alone than with chocolate, regarding time, temperature, and percentage of nonfat dry milk in the extraction buffer. In chocolate with peanut foods, the total Ara h 1 values were 10-fold higher than when products were extracted with phosphate buffer alone and could be up to 400-fold higher for individual foods. The dramatic improvement of Ara h 1 extraction should allow specific allergen monitoring in chocolate-containing food products and assessment of Ara h 1 exposure.

L22 ANSWER 4 OF 21 MEDLINE on STN
2004185497. PubMed ID: 15080811. Relevance of **Ara h1**, Ara h2 and Ara h3 in peanut-allergic patients, as determined by immunoglobulin E Western blotting, basophil-histamine release and intracutaneous testing: Ara h2 is the most important **peanut allergen.** Koppelman S J; Wensing M; Ertmann M; Knulst A C; Knol E

F. (TNO Nutrition and Food Research, Zeist, The Netherlands.. koppelman@voeding.tno.nl) . Clinical and experimental allergy : journal of the British Society for Allergy and Clinical Immunology, (2004 Apr) 34 (4) 583-90. Journal code: 8906443. ISSN: 0954-7894. Pub. country: England: United Kingdom. Language: English.

AB BACKGROUND: A number of allergenic proteins in peanut has been described and the relative importance of these allergens is yet to be determined. OBJECTIVES: We have investigated the relevance of previously identified **peanut allergens** in well-characterized peanut-allergic patients by in vitro, ex vivo and in vivo assays. METHODS: Thirty-two adult peanut-allergic patients were included based on careful and standardized patient history and the presence of peanut-specific IgE. The diagnosis peanut allergy was confirmed using double-blind placebo-controlled food challenges in 23 patients. Major **peanut allergens Ara h1**, Ara h2 and Ara h3 were purified from peanuts using ion-exchange chromatography. IgE immunoblotting was performed and IgE-cross-linking capacity was examined by measuring histamine release (HR) after incubating patient basophils as well as passively sensitized basophils with several dilutions of the allergens. Intracutaneous tests (ICTs) using 10-fold dilution steps of the purified allergens and crude peanut extract were performed. RESULTS: Ara h2 was recognized most frequently (26 out of 32) in all tests and induced both positive skin tests and basophil degranulation at low concentrations, whereas **Ara h1** and Ara h3 were recognized less frequently and reacted only at 100-fold higher concentrations as analysed with HR and intracutaneous testing (ICT). Next to the three tested allergens, proteins with molecular weights of somewhat smaller than 15 kDa were identified as a IgE-binding proteins on immunoblot in the majority of the patients (20 out of 32). CONCLUSION: We conclude that Ara h2 is, for our patient group, the most important **peanut allergen**, and that previously unidentified peanut proteins with molecular weights of somewhat smaller than 15 kDa may be important allergens as well. ICT in combination with basophil-HR and IgE immunoblotting provides insight in the patient specificity towards the individual **peanut allergens.**

L22 ANSWER 5 OF 21 EMBASE COPYRIGHT 2005 ELSEVIER INC. ALL RIGHTS RESERVED. on STN

2003438358 EMBASE Structure and organization of the genomic clone of a major **peanut allergen** gene, Ara h 1. Viquez O.M.; Konan K.N.; Dodo H.W.. O.M. Viquez, Food Biotechnology Laboratory, Dept. of Food and Animal Sciences, Alabama A and M University, Normal, AL 35762, United States. oviquez@aamu.edu. Molecular Immunology Vol. 40, No. 9, pp. 565-571 2003.
Refs: 45.
ISSN: 0161-5890. CODEN: IMCHAZ
Pub. Country: United Kingdom. Language: English. Summary Language: English.

ED Entered STN: 20031120

AB Peanut is one of the most allergenic foods. It contains multiple seed storage proteins identified as allergens, which are responsible for triggering IgE-mediated allergic reactions. Ara h 1 is a major **peanut allergen** recognized by over 90% of peanut sensitive population. The objectives of this study were to isolate, sequence, and determine the structure and organization of at least one genomic clone encoding Ara h 1. Two 100bp oligonucleotides were synthesized and used as probes to screen a peanut genomic library constructed in a Lambda FIX II vector. After three rounds of screening, four putative positive clones were selected and their DNA digested with SacI. A unique 12-13kb insert fragment was released, confirmed positive by Southern hybridization, subcloned into a pBluescript vector, and sequenced. Sequence analysis revealed a full-length Ara h 1 gene of 4447bp with four exons of 721, 176, 81 and 903bp and three introns of 71, 249 and 74bp. The deduced amino acid encodes a protein of 626 residues that is identical to the Ara h 1 cDNA clone P41b. Several well

characterized elements for promoter strength were found in the promoter region of Ara h 1 and include two TATA-boxes (TATATAAATA and TTATATATAT) at positions -89 and -348, respectively; a CAAT-box (CAAT) at position -133, a GC-box (CGGGACCGGGCCGG GCCTTCGGGCCGGGCCGGGT) at position -475, two G-boxes (TAACACGTACAC and ATGGACGTGAAA) at positions -264 and -1808, respectively; two RY elements (CATGCAC and CATGCAT) at positions -235 and -278, respectively; and other cis-element sequences. In the 3' UTR, a poly-A signal (AATAAA) was found at +2350, two additional stop codons (TAA) at +2303 and +2306, and TTTG/CTA/G motifs. Three introns and a potentially strong promoter could explain the high expression of the Ara h 1 gene. Amino acid sequence comparisons revealed high sequence similarity with other plant vicilins, member of the cupin superfamily.

L22 ANSWER 6 OF 21 BIOSIS COPYRIGHT (c) 2005 The Thomson Corporation on STN
2003:359679 Document No.: PREV200300359679. Binding of IgE from peanut allergic sera to the major **peanut allergens**, **Ara h1** and Ara h2: Comparison of IgE immunoblots to a functional assay. Palmer, G. W. [Reprint Author]; Dibbern, D. A. Jr.; Burks, A. W.; Bannon, G. A.; Bock, S. A.; Dreskin, S. C.. Private Practice, Mystic, CT, USA. Journal of Allergy and Clinical Immunology, (February 2003) Vol. 111, No. 2 Abstract Supplement, pp. S246. print. Meeting Info.: AAAAI 60th Anniversary Meeting. Denver, CO, USA. March 07-12, 2003. American Academy of Allergy, Asthma and Immunology. CODEN: JACIBY. ISSN: 0091-6749. Language: English.

L22 ANSWER 7 OF 21 BIOSIS COPYRIGHT (c) 2005 The Thomson Corporation on STN
2003:347651 Document No.: PREV200300347651. Relevance of **Ara h1**, Ara h2, and Ara h3 in peanut allergic patients, as determined by IgE-western-blotting, basophil histamine release, and intracutaneous testing: Ara h2 is the most important **peanut allergen.** Knol, E. F. [Reprint Author]; Wensing, M. [Reprint Author]; Vlooswijk, R.; Ertmann, M. [Reprint Author]; Knulst, A. C. [Reprint Author]; Koppelman, S. J.. Dermatology/Allergology, University Medical Center Utrecht, Utrecht, Netherlands. Journal of Allergy and Clinical Immunology, (February 2003) Vol. 111, No. 2 Abstract Supplement, pp. S194-S195. print. Meeting Info.: AAAAI 60th Anniversary Meeting. Denver, CO, USA. March 07-12, 2003. American Academy of Allergy, Asthma and Immunology. CODEN: JACIBY. ISSN: 0091-6749. Language: English.

L22 ANSWER 8 OF 21 SCISEARCH COPYRIGHT (c) 2005 The Thomson Corporation on STN
2003:607975 The Genuine Article (R) Number: 698QQ. Activated charcoal forms non-IgE binding complexes with peanut proteins. Vadas P (Reprint); Perelman B. Univ Toronto, St Michaels Hosp, Dept Med, Div Clin Immunol & Allergy, 30 Bond St, Toronto, ON M5B 1W8, Canada (Reprint); Univ Toronto, St Michaels Hosp, Dept Med, Div Clin Immunol & Allergy, Toronto, ON M5B 1W8, Canada. JOURNAL OF ALLERGY AND CLINICAL IMMUNOLOGY (JUL 2003) Vol. 112, No. 1, pp. 175-179. Publisher: MOSBY, INC. 11830 WESTLINE INDUSTRIAL DR, ST LOUIS, MO 63146-3318 USA. ISSN: 0091-6749. Pub. country: Canada. Language: English.
ABSTRACT IS AVAILABLE IN THE ALL AND IALL FORMATS

AB Background: Conventional management of peanut-induced anaphylaxis is composed of administration of epinephrine, antihistamine, and steroid and stabilization of airway, ventilatory, and circulatory function. Therapies directed toward slowing or preventing further absorption of peanut protein from the gastrointestinal tract after accidental ingestion have not been a routine part of management.

Objective: The purpose of this study was to determine the ability of activated charcoal to complex with peanut protein, thereby preventing its binding to either peanut-specific IgE or peanut-specific IgG.

Methods: Peanut protein was coincubated with micronized activated charcoal suspension at pH 3.5 or 7.4. Peanut protein complexed with charcoal was removed by centrifugation. Binding of residual peanut protein to peanut-specific IgG was measured by a sandwich ELISA assay. Also,

ability of uncomplexed peanut protein to bind to peanut-specific IgE was determined by Western blot and by skin prick testing in subjects with peanut allergy.

Results: Activated charcoal (AC) formed complexes with peanut protein, effectively competing for binding with peanut-specific IgG in a sandwich ELISA assay. AC complexed efficiently with peanut protein at both neutral and acidic pH in as little as 60 seconds. AC was also able to remove IgE-binding **peanut allergens** from solution as determined by Western blot and by skin prick testing in subjects with peanut allergy. A ratio of 200 mg of AC to 1 mg peanut protein was required for complete removal of peanut protein from solution. AC was able to complex with peanut protein within food matrices such as ice cream and chocolate.

Conclusion: The data presented herein show that AC removes both IgE-binding and IgG-binding peanut proteins from solution rapidly at both neutral and acidic pH. These data suggest that administration of AC may be useful as an adjunct to slow or to prevent further absorption of peanut protein from the gastrointestinal tract after accidental ingestion by individuals with peanut allergy.

L22 ANSWER 9 OF 21 CAPLUS COPYRIGHT 2005 ACS on STN

2003:585917 Document No. 139:259861 Persistent protective effect of heat-killed Escherichia coli producing "engineered," recombinant peanut proteins in a murine model of peanut allergy. Li, Xiu-Min; Srivastava, Kamal; Grishin, Alexander; Huang, Chih-Kang; Schofield, Brian; Burks, Wesley; Sampson, Hugh A. (Department of Pediatrics, Mount Sanai School of Medicine, New York, NY, 10029, USA). Journal of Allergy and Clinical Immunology, 112(1), 159-167 (English) 2003. CODEN: JACIBY. ISSN: 0091-6749. Publisher: Mosby, Inc..

AB Background: Peanut allergy (PNA) is a life-threatening food allergy for which there is no definitive treatment. Objective: the authors investigated the long-term immunomodulatory effect of heat-killed Escherichia coli producing engineered (mutated) **Ara h1**, 2, and 3 (HKE-MP123) administered rectally (pr) in a murine model of PNA. Methods: Peanut-allergic C3H/HeJ mice received 0.9 (low dose), 9 (medium dose), or 90 (high dose) μg HKE-MP123 pr, HKE-containing vector (HKE-V) alone, or vehicle alone (sham) weekly for 3 wk. Mice were challenged 2 wk later. A second and third challenge were performed at 4-wk intervals. Results: After the first challenge, all 3 HKE-MP123 and HKE-V-treated groups exhibited reduced symptom scores (.01,.05,.05, resp.) compared with the sham-treated group. Interestingly, only the medium- and high-dose HKE-MP123-treated mice remained protected for up to 10 wk after treatment accompanied by a significant reduction of plasma histamine levels compared with sham-treated mice (and.01, resp.). IgE levels were significantly lower in all HKE-MP123-treated groups, being most reduced in the high-dose HKE-MP123-treated group at the time of each challenge. IL-4, IL-13, IL-5, and IL-10 production by splenocytes of high-dose HKE-MP123-treated mice were significantly decreased (.001,.001, and.001, resp.), and IFN-γ and TGF-β production were significantly increased (and.01, resp.) compared with sham-treated mice at the time of the last challenge. Conclusions: Treatment with pr HKE-MP123 can induce long-term "down-regulation" of peanut hypersensitivity, which might be secondary to decreased antigen-specific TH2 and increased TH1 and T regulatory cytokine production

L22 ANSWER 10 OF 21 BIOSIS COPYRIGHT (c) 2005 The Thomson Corporation on STN

2003:209478 Document No.: PREV200300209478. Polyisotypic antibody and mixed cytokine responses to the major **peanut allergens** in an oral mouse food allergy model. van Wijk, F. [Reprint Author]; Hartgring, S. [Reprint Author]; Knippels, L. M.; Pieters, R. [Reprint Author]. Immunotoxicology, IRAS, Utrecht, Netherlands. Toxicological Sciences, (March 2003) Vol. 72, No. S-1, pp. 51. print.
Meeting Info.: 42nd Annual Meeting of the Society of Toxicology. Salt Lake City, Utah, USA. March 09-13, 2003. Society of Toxicology.

ISSN: 1096-6080 (ISSN print). Language: English.

L22 ANSWER 11 OF 21 BIOSIS COPYRIGHT (c) 2005 The Thomson Corporation on STN

2002:301849 Document No.: PREV200200301849. Relative potency of the major **peanut allergens**, **Ara h1** and Ara h2, in a functional assay. Palmer, G. William [Reprint author]; Dibbern, Donald A. [Reprint author]; Burks, Wesley; Bannon, Gary A.; Bock, S. Allan; Dreskin, Stephen C. [Reprint author]. University of Colorado, Denver, CO, USA. Journal of Allergy and Clinical Immunology, (January, 2002) Vol. 109, No. 1 Supplement, pp. S300. print.
Meeting Info.: 58th Annual Meeting of the American Academy of Allergy, Asthma and Immunology. New York, NY, USA. March 01-06, 2002. American Academy of Allergy, Asthma, and Immunology.
CODEN: JACIBY. ISSN: 0091-6749. Language: English.

L22 ANSWER 12 OF 21 BIOSIS COPYRIGHT (c) 2005 The Thomson Corporation on STN

2002:301398 Document No.: PREV200200301398. Immunotherapy for peanut allergy using modified allergens and a bacterial adjuvant. Stanley, Joseph Steve [Reprint author]; Buzen, Fred [Reprint author]; Cockrell, Gael [Reprint author]; West, Mike [Reprint author]; Srivastava, Kamal D.; Li, X. M.; Sampson, Hugh A.; Burks, Wesley [Reprint author]; Bannon, Gary A. [Reprint author]. University of Arkansas, Little Rock, AR, USA. Journal of Allergy and Clinical Immunology, (January, 2002) Vol. 109, No. 1 Supplement, pp. S93. print.
Meeting Info.: 58th Annual Meeting of the American Academy of Allergy, Asthma and Immunology. New York, NY, USA. March 01-06, 2002. American Academy of Allergy, Asthma, and Immunology.
CODEN: JACIBY. ISSN: 0091-6749. Language: English.

L22 ANSWER 13 OF 21 CAPLUS COPYRIGHT 2005 ACS on STN

2001:380762 Document No. 135:1229 Isolation and sequence of a full length genomic clone for allergen Ara h2 and down-regulation and silencing of allergen genes in transgenic peanut seeds. Dodo, Hortense W.; Arntzen, Charles J.; Konan, Koffi N'da; Viquez, Olga M. (Alabama A + M University, USA). PCT Int. Appl. WO 2001036621 A2 20010525, 73 pp. DESIGNATED STATES: W: AE, AG, AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, BZ, CA, CH, CN, CR, CU, CZ, DE, DK, DM, DZ, EE, ES, FI, GB, GD, GE, GH, GM, HR, HU, ID, IL, IN, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MA, MD, MG, MK, MN, MW, MX, MZ, NO, NZ, PL, PT, RO, RU, SD, SE, SG, SI, SK, SL, TJ, TM, TR, TT, TZ, UA, UG, US, UZ, VN, YU, ZA, ZW, AM, AZ, BY, KG, KZ, MD, RU, TJ, TM; RW: AT, BE, BF, BJ, CF, CG, CH, CI, CM, CY, DE, DK, ES, FI, FR, GA, GB, GR, IE, IT, LU, MC, ML, MR, NE, NL, PT, SE, SN, TD, TG, TR. (English). CODEN: PIXXD2. APPLICATION: WO 2000-US31657 20001120. PRIORITY: US 1999-PV167255 19991119.

AB An allergen-free transgenic peanut seed is produced by recombinant methods. Peanut plants are transformed with multiple copies of each of the allergen genes, or fragments thereof, to suppress gene expression and allergen protein production Alternatively, peanut plants are transformed with **peanut allergen** antisense genes introduced into the peanut genome as antisense fragments, sense fragments, or combinations of both antisense and sense fragments. Peanut transgenes are under the control of the 35S promoter, or the promoter of the Ara h2 gene to produce antisense RNAs, sense RNAs, and double-stranded RNAs for suppressing allergen protein production in peanut plants. A full length genomic clone for allergen Ara h2 is isolated and sequenced. The ORF is 622 nucleotides long. The predicted encoded protein is 207 amino acids long and includes a putative transit peptide of 21 residues. One polyadenilation signal is identified at position 951. Six addnl. stop codons are observed A promoter region was revealed containing a putative TATA box located at position -72. Homologous regions were identified between Ara h2, h6, and h7, and between Ara h3 and h4, and between Ara h1P41B and Ara h1P17. The homologous regions will be used for the screening of peanut genomic library to isolate all **peanut allergen** genes and for

down-regulation and silencing of multiple **peanut allergen** genes.

L22 ANSWER 14 OF 21 CAPLUS COPYRIGHT 2005 ACS on STN
2001:295491 Document No. 135:151859 Detection of **peanut allergens** in breast milk of lactating women. Vadas, Peter; Wai, Yvonne; Burks, Wesley; Perelman, Boris (Division of Allergy and Clinical Immunology, St Michael's Hospital, University of Toronto, Toronto, ON, Can.). JAMA, the Journal of the American Medical Association, 285(13), 1746-1748 (English) 2001. CODEN: JAMAAP. ISSN: 0098-7484. Publisher: American Medical Association.
AB Most individuals who react to peanuts do so on their 1st known exposure. A potential but unproven route of occult exposure resulting in sensitization to peanut is via breast milk during lactation. To investigate the ability of maternal dietary peanut protein to pass into breast milk during lactation. Clin. investigation conducted at 2 North American hospitals from Mar. 1999 to Oct. 2000 including 23 healthy, lactating women aged 21-35 yr. Each woman consumed 50 g of dry roasted peanuts, after which breast milk samples were collected at hourly intervals. Presence in breast milk of total peanut protein, analyzed by a sandwich ELISA, and 2 major **peanut allergens**, Ara h 1 and Ara h 2, detected by immunoblot anal. Peanut protein was detected in 11 of 23 subjects. It was detected in 10 subjects within 2 h of ingestion and in 1 subject within 6 h. The median peak peanut protein concentration in breast milk was 200 ng/mL (mean, 222 ng/mL; range, 120-430 ng/mL). Both major **peanut allergens** Ara h 1 and Ara h 2 were detected. Conclusions Peanut protein is secreted into breast milk of lactating women following maternal dietary ingestion. Exposure to peanut protein during breastfeeding is a route of occult exposure that may result in sensitization of at-risk infants.

L22 ANSWER 15 OF 21 MEDLINE on STN
2001435540. PubMed ID: 11328490. Hypogin, a novel antifungal peptide from peanuts with sequence similarity to **peanut allergen.**
Ye X Y; Ng T B. (Department of Biochemistry, Faculty of Medicine, Chinese University of Hong Kong, Shatin, Hong Kong, China.) journal of peptide research : official journal of the American Peptide Society, (2001 Apr) 57 (4) 330-6. Journal code: 9707067. ISSN: 1397-002X. Pub. country: Denmark. Language: English.
AB A protein designated hypogin, with a prominent suppressive action on the growth of the fungi Mycosphaerella arachidicola, Fusarium oxysporum and Coprinus comatus, was isolated from seeds of the peanut Arachis hypogaea. The protein inhibited human immunodeficiency virus (HIV) reverse transcriptase and enzymes associated with HIV infection including alpha-glucosidase and beta-glucosidase. The proliferative response of mouse splenocytes was attenuated in the presence of the protein. The protein exhibited a molecular mass of 7.2 kDa in tricine gel electrophoresis and gel filtration on Superdex 75 and an N-terminal sequence resembling **peanut allergen Ara H1.** The isolation procedure involved affinity chromatography on Affi-gel blue gel and ion-exchange chromatography on CM-Sepharose. The protein was adsorbed in both chromatographic media.

L22 ANSWER 16 OF 21 CAPLUS COPYRIGHT 2005 ACS on STN
2001:408584 Document No. 135:45362 Peanut (Arachis hypogaea) - a common cause of food allergy. Wroblewska, Barbara; Jedrychowski, Lucjan (Inst. Rozrodu Zwierzat i Badan Zywnosci, Polska Akademia Nauk, Olsztyn, 10-747, Pol.). Zywnosc, 7(4), 104-113 (Polish) 2000. CODEN: ZYWNFL. Publisher: Polskie Towarzystwo Technologow Zywnosci, Oddzial Malopolski.
AB The Ridascreen Peanut sandwich ELISA test (R-Biopharm) assay kit was used to determine the main **peanut allergen Ara h1** content in 9 samples of raw plant material from peanuts (Arachis hypogaea), almonds (Amygdalus communis), soybean (Glycine max), oats, barley, wheat, buckwheat (Fagopyrum sagittatum), walnuts (Juglans regia), hazelnuts (Corylus avellana) and in 10 samples of sweet peanut

confectionery (chocolate products, chocolate bars, sweet cream spreads). The assay cross-reactivity was examined The **Ara h1** allergen was found in all sweet confectionery samples, even in those that were not labeled to contain peanuts. Three raw materials (almonds, soybeans, oats) cross-reacted with the **Ara h1** antibody.

L22 ANSWER 17 OF 21 CAPLUS COPYRIGHT 2005 ACS on STN
2002:277584 Document No. 136:368007 Peanut allergy: From allergens to immunotherapy. Helm, Ricki M.; Sampson, Hugh A.; Bannon, Gary A.; Burks, A. Wesley (Arkansas Children's Hospital, University of Arkansas for Medical Sciences, Little Rock, AR, 72202, USA). Recent Research Developments in Allergy & Clinical Immunology, 1, 11-21 (English) 2000. CODEN: RRDACJ. Publisher: Research Signpost.
AB A review. Peanut hypersensitivity represents a significant health problem to the peanut sensitive population because of the potential severity, life-long nature, and ubiquitous use of peanut products. The relevant allergens have been identified using serum IgE from peanut-sensitive individuals using physicochem. and immunol. procedures. Ara h 1 and 2 represent major allergens that bind IgE from greater than 90% of the sensitive population. Ara h 3 and other **peanut allergens** bind serum IgE from less than 50% of the sensitive population. Major linear IgE-binding epitopes have been identified for each of the allergens using recombinant proteins from a cDNA expression library. Mutational anal. of epitopes by alanine substitution indicates that a single amino acid change results in loss of IgE binding. Ara h 1 and 2 are being used to investigate the potential application of vaccine development in the modulation of food allergy. Results from these investigations will allow for the design of new diagnostic and immunotherapeutic approaches to peanut hypersensitivity.

L22 ANSWER 18 OF 21 CAPLUS COPYRIGHT 2005 ACS on STN
1998:383080 Document No. 129:121594 Biochemical and structural analysis of the IgE binding sites on **Ara h1**, an abundant and highly allergenic peanut protein. Shin, David S.; Compadre, Cesar M.; Maleki, Soheila J.; Kopper, Randall A.; Sampson, Hugh; Huang, Shau K.; Burks, A. Wesley; Bannon, Gary A. (Department of Biochemistry & Molecular Biology, Arkansas Children's Hospital, University of Arkansas for Medical Sciences, Little Rock, AR, 72205, USA). Journal of Biological Chemistry, 273(22), 13753-13759 (English) 1998. CODEN: JBCHA3. ISSN: 0021-9258. Publisher: American Society for Biochemistry and Molecular Biology.
AB Allergy to peanut is a significant IgE-mediated health problem because of the high prevalence, potential severity, and chronicity of the reaction. **Ara h1**, an abundant peanut protein, is recognized by serum IgE from >90% of peanut-sensitive individuals. It has been shown to belong to the vicilin family of seed storage proteins and to contain 23 linear IgE binding epitopes. Here, the authors determined the critical amino acids within each of the IgE binding epitopes of **Ara h1** that are important for Ig binding. Surprisingly, substitution of a single amino acid within each of the epitopes led to loss of IgE binding. In addition, hydrophobic residues appeared to be most critical for IgE binding. The position of each of the IgE binding epitopes on a homol.-based mol. model of **Ara h1** showed that they were clustered into 2 main regions, despite their more even distribution in the primary sequence. Finally, the authors have shown that **Ara h1** forms a stable trimer by the use of a reproducible fluorescence assay. This information will be important in studies designed to reduce the risk of peanut-induced anaphylaxis by lowering the IgE binding capacity of the allergen.

L22 ANSWER 19 OF 21 MEDLINE on STN
1998339794. PubMed ID: 9677140. Identification and partial characterization of multiple major allergens in peanut proteins. de Jong E C; Van Zijverden M; Spanhaak S; Koppelman S J; Pellegrom H; Penninks A H. (TNO Nutrition and Food Research Institute, Immunotoxicology group, Zeist,

The Netherlands.) Clinical and experimental allergy : journal of the British Society for Allergy and Clinical Immunology, (1998 Jun) 28 (6) 743-51. Journal code: 8906443. ISSN: 0954-7894. Pub. country: ENGLAND: United Kingdom. Language: English.

AB BACKGROUND: Peanuts are a major cause of food allergies both in children as in adults which can induce an anaphylactic shock. The identification and characterization of **peanut allergens** could lead to more insight into the mechanism and contribute to the improvement of diagnostic tests and treatment for peanut allergy. OBJECTIVE: In the present study, the peanut protein-specific immunoglobulin concentrations as well as their recognition of the various peanut proteins or protein subunits was determined in the plasma of peanut-allergic (PA) and non-allergic (NA) individuals. Moreover, two **peanut allergens** were characterized in more detail to confirm them as the earlier described **Ara h1** and Ara h2. METHODS: The presence of Ig-binding sites in peanut proteins was studied by immunoblotting assays whereas the concentrations of peanut-specific Ig was determined by ELISA. RESULTS: Peanut proteins were found to contain multiple binding sites for immunoglobulins. Of these proteins, six were recognized by peanut-specific IgE present in more than 50% of the plasma samples of the PA group. Their molecular weights were approximately 44, 40, 33, 21, 20 and 18 kDa. The last three protein bands were recognized by peanut-specific IgE present in more than 70% of the PA plasma samples and were thought to contain Ara h2. This allergen as well as another protein that was thought to be **Ara h1**, which was not recognized by the majority of the patients' IgE-containing plasma samples, were isolated and the N terminal amino acid sequence was determined. Peanut protein-specific IgA, IgM, IgG and IgG-subclasses showed a more diverse recognition pattern of peanut protein in the PA group compared to the NA group. No differences were found in the plasma concentrations of peanut protein-specific immunoglobulins of the various classes between the PA and NA group. CONCLUSIONS: From the present study, we conclude that peanuts contain multiple allergens, of which six can be described as major allergens, Ara h2 included. In our population **Ara h1** is not a major allergen. The recognition of peanut proteins by immunoglobulins is more diverse in PA individuals compared with NA individuals which, however, is not substantiated in the concentrations of peanut-specific immunoglobulins in plasma, other than IgE.

L22 ANSWER 20 OF 21 BIOSIS COPYRIGHT (c) 2005 The Thomson Corporation on STN

1997:220385 Document No.: PREV199799512101. Mapping and mutational analysis of the IgE-binding epitopes on Ara h 1, a legume vicilin protein and a major allergen in peanut hypersensitivity. Burks, A. Wesley; Shin, David; Cockrell, Gael; Stanley, J. Steven; Helm, Ricki M.; Bannon, Gary A. [Reprint author]. Univ. Arkansas Med. Sci., Slot 516, 4301 W. Markham, Little Rock, AR 72205, USA. European Journal of Biochemistry, (1997) Vol. 245, No. 2, pp. 334-339.
CODEN: EJBCAI. ISSN: 0014-2956. Language: English.

AB Peanut allergy is a significant health problem because of the prevalence and potential severity of the allergic reaction. Serum IgE from patients with documented peanut hypersensitivity reactions and overlapping peptides were used to identify the IgE-binding epitopes on the major **peanut allergen**, Ara h 1. At least twenty-three different linear IgE-binding epitopes, located throughout the length of the Ara h 1 protein, were identified. All of the epitopes were 6-10 amino acids in length, but there was no obvious sequence motif shared by all peptides. Four of the peptides appeared to be immunodominant IgE-binding epitopes in that they were recognized by serum from more than 80% of the patients tested and bound more IgE than any of the other Ara h 1 epitopes. Mutational analysis of the immunodominant epitopes revealed that single amino acid changes within these peptides had dramatic effects on IgE-binding characteristics. The identification and determination of the IgE-binding capabilities of core amino acids in epitopes on the Ara h 1 protein will make it possible to address the pathophysiologic and

immunologic mechanisms regarding peanut hypersensitivity reactions specifically and food hypersensitivity in general.

L22 ANSWER 21 OF 21 CAPLUS COPYRIGHT 2005 ACS on STN
1996:565773 Document No. 125:219989 Reinvestigation of the major **peanut allergen** Ara h 1 on the molecular level.
Buschmann, Liselotte; Petersen, Arnd; Schlaak, Max; Becker, Wolf-Meinhard (Division Allergology, Research Institute Borstel, Germany). Monographs in Allergy, 32(Highlights in Food Allergy), 92-98 (English) 1996. CODEN: MOALAR. ISSN: 0077-0760. Publisher: Karger.
AB Studies on **peanut allergens** resulted in some inconsistent findings. Thus, the authors established monoclonal antibodies directed against peanut extract aimed at the 66-kD allergen which was identified as a major allergen, Ara h 1. The monoclonal antibody PN-t was found to be species-specific with a reactivity to this protein. In this study, the antigenic and allergenic structures of **peanut allergens** were reinvestigated by 2D-PAGE with the aid of patients' sera, monoclonal antibodies, and lectins. The main reactivity of IgE was found in the 66-kD region. The Ara h 1 allergen was identified as Con A (Con A)-reactive glycoprotein. Amino acid composition and sequence studies showed that Ara h 1 belongs to vicilins. Ara h 1 consists of at least 16 isoallergens , and at least 2 of the isoforms slightly differ in mol. weight Moreover, Ara h 1 forms dimers and trimers of the isoallergens. It was concluded that Ara h 1 and the Con A-reactive allergen described by Barnett D. and Howden MEH (1986) are identical.

=> s Ara h2
L23 53 ARA H2

=> s l23 and modified
L24 2 L23 AND MODIFIED

=> dup remove l24
PROCESSING COMPLETED FOR L24
L25 2 DUP REMOVE L24 (0 DUPLICATES REMOVED)

=> d l25 1-2 cbib abs

L25 ANSWER 1 OF 2 BIOSIS COPYRIGHT (c) 2005 The Thomson Corporation on STN
2002:301398 Document No.: PREV200200301398. Immunotherapy for peanut allergy using **modified** allergens and a bacterial adjuvant. Stanley, Joseph Steve [Reprint author]; Buzen, Fred [Reprint author]; Cockrell, Gael [Reprint author]; West, Mike [Reprint author]; Srivastava, Kamal D.; Li, X. M.; Sampson, Hugh A.; Burks, Wesley [Reprint author]; Bannon, Gary A. [Reprint author]. University of Arkansas, Little Rock, AR, USA. Journal of Allergy and Clinical Immunology, (January, 2002) Vol. 109, No. 1 Supplement, pp. S93. print.
Meeting Info.: 58th Annual Meeting of the American Academy of Allergy, Asthma and Immunology. New York, NY, USA. March 01-06, 2002. American Academy of Allergy, Asthma, and Immunology.
CODEN: JACIBY. ISSN: 0091-6749. Language: English.

L25 ANSWER 2 OF 2 CAPLUS COPYRIGHT 2005 ACS on STN
1996:612305 Document No. 125:273253 Stability of food allergens to digestion in vitro. Astwood, James D.; Leach, John N.; Fuchs, Roy L. (Monsanto Co., St Louis, MO, 63198, USA). Nature Biotechnology, 14(10), 1269-1273 (English) 1996. CODEN: NABIF9. ISSN: 1087-0156. Publisher: Nature Publishing Co..
AB An integral part of the safety assessment of genetically **modified** plants is consideration of possible human health effects, especially food allergy. Prospective testing for allergenicity of proteins obtained from sources with no prior history of causing allergy has been difficult because of the absence of valid methods and models. Food allergens may share physicochem. properties that distinguish them from nonallergens,

properties that may be used as a tool to predict the inherent allergenicity of proteins newly introduced into the food supply by genetic engineering. One candidate property is stability to digestion. We have systematically evaluated the stability of food allergens that are active via the gastrointestinal tract in a simple model of gastric digestion, emphasizing the major allergens of plant-derived foods such as legumes (peanuts and soybean). Important food allergens were stable to digestion in the gastric model (simulated gastric fluid). For example, soybean β-conglycinin was stable for 60 min. In contrast, nonallergenic food proteins, such as spinach ribulose bis-phosphate carboxylase/oxygenase, were digested in simulated gastric fluid within 15 s. The data are consistent with the hypothesis that food allergens must exhibit sufficient gastric stability to reach the intestinal mucosa where absorption and sensitization (development of atopy) can occur. Thus, the stability to digestion is a significant and valid parameter that distinguishes food allergens from nonallergens.

=> dup remove l23
PROCESSING COMPLETED FOR L23
L26 32 DUP REMOVE L23 (21 DUPLICATES REMOVED)

=> d l26 1-32 cbib abs

L26 ANSWER 1 OF 32 MEDLINE on STN
2005181166. PubMed ID: 15813812. Peanut- and cow's milk-specific IgE, Th2 cells and local anaphylactic reaction are induced in Balb/c mice orally sensitized with cholera toxin. Adel-Patient K; Bernard H; Ah-Leung S; Creminon C; Wal J-M. (Laboratoire Inra d'Immuno-Allergie Alimentaire, CEA de Saclay, Gif sur Yvette cedex-France.) Allergy, (2005 May) 60 (5) 658-64. Journal code: 7804028. ISSN: 0105-4538. Pub. country: Denmark. Language: English.

AB Background: The development of animal models developing specific immunoglobulin (Ig)E presenting the same specificity as human IgE and similar clinical symptoms as those observed in allergic patients are of great interest for the understanding of mechanisms involved in the induction and regulation of food allergy. Methods: Balb/c female mice were sensitized with whole peanut protein extract (WPPE) by means of intraperitoneal (i.p.) injections with alum or gavages with cholera toxin (CT). The WPPE specific IgE, IgG1 and IgG2a were monitored. Th2 cells activation was analysed assaying interleukin (IL)-4 and IL-5 vs IFNgamma on reactivated splenocytes. Local anaphylactic reaction was evaluated by assaying histamine in faecal samples. The oral sensitization protocol was further extended to cow's milk proteins (CMP). Results: Balb/c mice developed high peanut-specific IgE and IgG1 responses either after i.p. or oral sensitizations. In both cases, antibodies were specific to polymer of glycinin fragments, containing polypeptides from Ara h3/4, and to a lesser extent to Ara h1 and **Ara h2.** Interleukin-4 and IL-5 production were evidenced. Balb/c mice could also be sensitized to CMP, as demonstrated by CMP-specific IL-4 and IL-5 secretions and induction of IgE specific for whole caseins, beta-lactoglobulin, serum bovine albumin and lactoferrin. Of interest was the occurrence of a local anaphylactic reaction in the peanut and CM models. Conclusions: In contrast with previous authors, Balb/c mice were sensitized and evidenced an allergic reaction after oral administrations of peanut or CMP plus CT, providing an interesting model for further studies on immunopathogenic mechanisms.

L26 ANSWER 2 OF 32 CAPLUS COPYRIGHT 2005 ACS on STN
2004:252189 Document No. 140:286142 Hybrid polypeptides comprising Ii-key motif and MHC class I or II-presented epitope of antigen, allergen or tumor antigen as vaccines against infection, allergy and cancer. Humphreys, Robert E.; Xu, Minzhen (Antigen Express, Inc., USA). U.S. Pat. Appl. Publ. US 2004058881 A1 20040325, 90 pp. (English). CODEN: USXXCO. APPLICATION: US 2002-253286 20020924.

AB Disclosed is a nucleic acid mol. comprising a first expressible sequence encoding a protein of interest or polypeptide of interest which contains an MHC Class II-presented epitope. In addition, the nucleic acid mol. comprises a second expressible nucleic acid sequence encoding an antigen presentation-enhancing hybrid polypeptide. The antigen presentation enhancing hybrid polypeptide includes the following elements: i) an N-terminal element consisting essentially of 4-16 residues of the mammalian Ii-Key peptide: LRMKLPKPPKPVSKMR and non-N-terminal deletion modifications thereof that retain antigen presentation enhancing activity; ii) a C-terminal element comprising an MHC Class II-presented epitope in the form of a polypeptide or peptidomimetic structure which binds to the antigenic peptide binding site of an MHC class II mol., the MHC Class II-presented epitope being contained in the protein of interest of step a); and iii) an intervening peptidyl structure linking the N-terminal and C-terminal elements of the hybrid, the peptidyl structure having a length of about 20 amino acids or less. Exemplified proteins are allergen: Ara h 1-3, Fel d 1, Phi p 1, Phl p 5a, Bla q 5, and bee venom phospholipase A2; tumor antigen: CEA, CA-125, PSA, gp100, Pmel17, TRP-2, melanoma tyrosinase, MART-1, and Her-2 neu; pathogenic antigen: anthrax toxin lethal factor, anthrax protective antigen, Variola virus B5R protein, and Ebola virus membrane-associated protein VP24; and autoantigen: myelin basic protein, proteolipid protein, and myelin-oligodendrocyte glycoprotein precursor.

L26 ANSWER 3 OF 32 MEDLINE on STN DUPLICATE 1

2004185497. PubMed ID: 15080811. Relevance of Ara h1, **Ara h2** and Ara h3 in peanut-allergic patients, as determined by immunoglobulin E Western blotting, basophil-histamine release and intracutaneous testing: **Ara h2** is the most important peanut allergen. Koppelman S J; Wensing M; Ertmann M; Knulst A C; Knol E F. (TNO Nutrition and Food Research, Zeist, The Netherlands.. koppelman@voeding.tno.nl) . Clinical and experimental allergy : journal of the British Society for Allergy and Clinical Immunology, (2004 Apr) 34 (4) 583-90. Journal code: 8906443. ISSN: 0954-7894. Pub. country: England: United Kingdom. Language: English.

AB BACKGROUND: A number of allergenic proteins in peanut has been described and the relative importance of these allergens is yet to be determined. OBJECTIVES: We have investigated the relevance of previously identified peanut allergens in well-characterized peanut-allergic patients by in vitro, ex vivo and in vivo assays. METHODS: Thirty-two adult peanut-allergic patients were included based on careful and standardized patient history and the presence of peanut-specific IgE. The diagnosis peanut allergy was confirmed using double-blind placebo-controlled food challenges in 23 patients. Major peanut allergens Ara h1, **Ara h2** and Ara h3 were purified from peanuts using ion-exchange chromatography. IgE immunoblotting was performed and IgE-cross-linking capacity was examined by measuring histamine release (HR) after incubating patient basophils as well as passively sensitized basophils with several dilutions of the allergens. Intracutaneous tests (ICTs) using 10-fold dilution steps of the purified allergens and crude peanut extract were performed. RESULTS: **Ara h2** was recognized most frequently (26 out of 32) in all tests and induced both positive skin tests and basophil degranulation at low concentrations, whereas Ara h1 and Ara h3 were recognized less frequently and reacted only at 100-fold higher concentrations as analysed with HR and intracutaneous testing (ICT). Next to the three tested allergens, proteins with molecular weights of somewhat smaller than 15 kDa were identified as a IgE-binding proteins on immunoblot in the majority of the patients (20 out of 32). CONCLUSION: We conclude that **Ara h2** is, for our patient group, the most important peanut allergen, and that previously unidentified peanut proteins with molecular weights of somewhat smaller than 15 kDa may be important allergens as well. ICT in combination with basophil-HR and IgE immunoblotting provides insight in the patient specificity towards the individual peanut allergens.

L26 ANSWER 4 OF 32 BIOSIS COPYRIGHT (c) 2005 The Thomson Corporation on STN
2004:209278 Document No.: PREV200400210128. Purification and IgE-binding properties of peanut allergen Ara h 6: Evidence for cross-reactivity with **Ara h2.** Koppelman, S. J. [Reprint Author]; De Jong, G. A. H. [Reprint Author]; Ertmann, M.; Wensing, M.; Hefle, S. L.; Knulst, A. C.; Knol, E. F.. Protein Technology, TNO Nutrition and Food Research, Zeist, Netherlands. Journal of Allergy and Clinical Immunology, (February 2004) Vol. 113, No. 2 Supplement, pp. S238. print.
Meeting Info.: 60th Annual Meeting of the American Academy of Allergy, Asthma and Immunology (AAAAI). San Francisco, CA, USA. March 19-23, 2004. American Academy of Allergy, Asthma and Immunology.
CODEN: JACIBY. ISSN: 0091-6749. Language: English.

L26 ANSWER 5 OF 32 BIOSIS COPYRIGHT (c) 2005 The Thomson Corporation on STN
2004:209261 Document No.: PREV200400210116. The role of CTLA-4 and a mucosal adjuvant cholera toxin in oral sensitization to peanut. van Wijk, F. [Reprint Author]; Hoeks, S. [Reprint Author]; Knippels, L.; Boon, L.; Koppelman, S.; Pieters, R. [Reprint Author]. Immunotoxicology, Institute for Risk Assessment Sciences, Utrecht, Netherlands. Journal of Allergy and Clinical Immunology, (February 2004) Vol. 113, No. 2 Supplement, pp. S234-S235. print.
Meeting Info.: 60th Annual Meeting of the American Academy of Allergy, Asthma and Immunology (AAAAI). San Francisco, CA, USA. March 19-23, 2004. American Academy of Allergy, Asthma and Immunology.
CODEN: JACIBY. ISSN: 0091-6749. Language: English.

L26 ANSWER 6 OF 32 CAPLUS COPYRIGHT 2005 ACS on STN
2004:814893 Document No. 142:73611 Isoforms of the Major Peanut Allergen Ara h 2: IgE Binding in Children with Peanut Allergy. Hales, Belinda J.; Bosco, Anthony; Mills, Kristina L.; Hazell, Lee A.; Loh, Richard; Holt, Patrick G.; Thomas, Wayne R. (Centre for Child Health Research, Telethon Institute for Child Health Research, University of Western Australia, West Perth, Australia). International Archives of Allergy and Immunology, 135(2), 101-107 (English) 2004. CODEN: IAAIEG. ISSN: 1018-2438. Publisher: S. Karger AG.
AB The major peanut allergen Ara h 2 consists of 2 isoforms, namely Ara h 2.0101 and Ara h 2.0201. The recently identified Ara h 2.0201 isoform contains an extra 12 amino acids including an extra copy of the reported immunodominant epitope DPYSPS. This study aimed to evaluate the IgE binding of the 2 Ara h 2 isoforms. Ten clones of Ara h 2 were sequenced to assess the relative frequency of the Ara h 2 isoforms and to identify whether there was further variation in the Ara h 2 sequence. IgE binding to Ara h 2.0101 and Ara h 2.0201 was measured for 70 peanut-allergic children using an IgE DELFIA assay to quantitate specific IgE binding. A competition assay was used to measure whether Ara h 2.0201 contained IgE epitopes other than those found for Ara h 2.0101. The original Ara h 2.0101 sequence was found for 6/10 clones and Ara h 2.0201 was found for 2/10 clones. Ara h 2.0201 had the expected insertion of 12 amino acids as well as substitutions at positions 40 (40G) and 142 (142E). Two new isoforms were identified as different polymorphisms of position 142. One Ara h 2.01 clone (Ara h 2.0102) contained 142E and one Ara h 2.02 clone (Ara h 2.0202) contained 142D. A polymorphism that was previously identified by other investigators at position 77 (77Q or 77R) was not found for any of the 10 sequences. Although the level of IgE binding to Ara h 2.0201 of individual patients was frequently higher than the binding to Ara h 2.0101, there was a strong correlation in binding to both isoforms and when analyzed as a group the means were similar. Ara h 2.0101 was not as efficient at blocking reactivity to Ara h 2.0201 indicating there is an addnl. IgE specificity for the Ara h 2.0201 isoform. Conclusions: Ara h 2.0201 has similar but higher IgE binding than the originally sequenced Ara h 2.0101 isoform and contains other IgE specificities.

L26 ANSWER 7 OF 32 BIOSIS COPYRIGHT (c) 2005 The Thomson Corporation on STN
2003:239426 Document No.: PREV200300239426. Silencing the three major

allergens for the production of hypoallergenic peanut. Konan, K. N. [Reprint Author]; Viquez, O. M. [Reprint Author]; Dodo, H. W. [Reprint Author]. Alabama A and M University, Normal, AL, USA. Journal of Allergy and Clinical Immunology, (April 2003) Vol. 111, No. 4, pp. 909. print. Meeting Info.: 60th Anniversary Meeting of the American Academy of Allergy, Asthma and Immunology (AAAAI). Denver, CO, USA. March 07-12, 2003. American Academy of Allergy, Asthma and Immunology. CODEN: JACIBY. ISSN: 0091-6749. Language: English.

L26 ANSWER 8 OF 32 BIOSIS COPYRIGHT (c) 2005 The Thomson Corporation on STN
2003:517960 Document No.: PREV200300521766. Detection of peanut using real-time polymerase chain reaction. Hird, H. [Reprint Author]; Lloyd, J.; Goodier, R.; Brown, J.; Reece, P.. Cellular and Molecular Sciences, Central Science Laboratory, Sand Hutton, York, North Yorkshire, YO41 1LZ, UK. h.hird@csl.gov.uk. European Food Research and Technology, (September 2003) Vol. 217, No. 3, pp. 265-268. print.
ISSN: 1438-2377 (ISSN print). Language: English.
AB Preliminary results are presented on a sensitive and robust assay for the identification of peanut in commercial products using real-time PCR technology. Peanut specific primers and probe, designed using the Arah 2 gene, were optimised for real-time PCR using an ABI PRISM 7700. Commercial extraction kits employing different technological strategies were assessed for the extraction of PCR quality peanut DNA template. The specificity of the primer and probe set was determined using a wide range of food items and the limit of detection and quantification calculated using dilutions of peanut DNA. The assay was used to detect spiked or trace level of peanut in commercial samples and was finally used to detect peanut in a biscuit prepared with 2 ppm of lightly roasted peanut powder.

L26 ANSWER 9 OF 32 BIOSIS COPYRIGHT (c) 2005 The Thomson Corporation on STN
2003:359679 Document No.: PREV200300359679. Binding of IgE from peanut allergic sera to the major peanut allergens, Ara h1 and **Ara h2**: Comparison of IgE immunoblots to a functional assay. Palmer, G. W. [Reprint Author]; Dibbern, D. A. Jr.; Burks, A. W.; Bannon, G. A.; Bock, S. A.; Dreskin, S. C.. Private Practice, Mystic, CT, USA. Journal of Allergy and Clinical Immunology, (February 2003) Vol. 111, No. 2 Abstract Supplement, pp. S246. print.
Meeting Info.: AAAAI 60th Anniversary Meeting. Denver, CO, USA. March 07-12, 2003. American Academy of Allergy, Asthma and Immunology.
CODEN: JACIBY. ISSN: 0091-6749. Language: English.

L26 ANSWER 10 OF 32 BIOSIS COPYRIGHT (c) 2005 The Thomson Corporation on STN
2003:347651 Document No.: PREV200300347651. Relevance of Ara h1, **Ara h2**, and Ara h3 in peanut allergic patients, as determined by IgE-western-blotting, basophil histamine release, and intracutaneous testing: **Ara h2** is the most important peanut allergen.
Knol, E. F. [Reprint Author]; Wensing, M. [Reprint Author]; Vlooswijk, R.; Ertmann, M. [Reprint Author]; Knulst, A. C. [Reprint Author]; Koppelman, S. J.. Dermatology/Allergology, University Medical Center Utrecht, Utrecht, Netherlands. Journal of Allergy and Clinical Immunology, (February 2003) Vol. 111, No. 2 Abstract Supplement, pp. S194-S195. print.
Meeting Info.: AAAAI 60th Anniversary Meeting. Denver, CO, USA. March 07-12, 2003. American Academy of Allergy, Asthma and Immunology.
CODEN: JACIBY. ISSN: 0091-6749. Language: English.

L26 ANSWER 11 OF 32 SCISEARCH COPYRIGHT (c) 2005 The Thomson Corporation on STN
2003:607975 The Genuine Article (R) Number: 698QQ. Activated charcoal forms non-IgE binding complexes with peanut proteins. Vadas P (Reprint); Perelman B. Univ Toronto, St Michaels Hosp, Dept Med, Div Clin Immunol & Allergy, 30 Bond St, Toronto, ON M5B 1W8, Canada (Reprint); Univ Toronto, St Michaels Hosp, Dept Med, Div Clin Immunol & Allergy, Toronto, ON M5B 1W8, Canada. JOURNAL OF ALLERGY AND CLINICAL IMMUNOLOGY (JUL 2003) Vol. 112, No. 1, pp. 175-179. Publisher: MOSBY, INC. 11830 WESTLINE INDUSTRIAL

DR, ST LOUIS, MO 63146-3318 USA. ISSN: 0091-6749. Pub. country: Canada. Language: English.
ABSTRACT IS AVAILABLE IN THE ALL AND IALL FORMATS

AB Background: Conventional management of peanut-induced anaphylaxis is composed of administration of epinephrine, antihistamine, and steroid and stabilization of airway, ventilatory, and circulatory function. Therapies directed toward slowing or preventing further absorption of peanut protein from the gastrointestinal tract after accidental ingestion have not been a routine part of management.

Objective: The purpose of this study was to determine the ability of activated charcoal to complex with peanut protein, thereby preventing its binding to either peanut-specific IgE or peanut-specific IgG.

Methods: Peanut protein was coincubated with micronized activated charcoal suspension at pH 3.5 or 7.4. Peanut protein complexed with charcoal was removed by centrifugation. Binding of residual peanut protein to peanut-specific IgG was measured by a sandwich ELISA assay. Also, ability of uncomplexed peanut protein to bind to peanut-specific IgE was determined by Western blot and by skin prick testing in subjects with peanut allergy.

Results: Activated charcoal (AC) formed complexes with peanut protein, effectively competing for binding with peanut-specific IgG in a sandwich ELISA assay. AC complexed efficiently with peanut protein at both neutral and acidic pH in as little as 60 seconds. AC was also able to remove IgE-binding peanut allergens from solution as determined by Western blot and by skin prick testing in subjects with peanut allergy. A ratio of 200 mg of AC to 1 mg peanut protein was required for complete removal of peanut protein from solution. AC was able to complex with peanut protein within food matrices such as ice cream and chocolate.

Conclusion: The data presented herein show that AC removes both IgE-binding and IgG-binding peanut proteins from solution rapidly at both neutral and acidic pH. These data suggest that administration of AC may be useful as an adjunct to slow or to prevent further absorption of peanut protein from the gastrointestinal tract after accidental ingestion by individuals with peanut allergy.

L26 ANSWER 12 OF 32 CAPLUS COPYRIGHT 2005 ACS on STN

2004:278417 Document No. 141:1944 Towards the development of a hypoallergenic peanut through genetic transformation. Konan, Koffi N.; Viquez, Olga M.; Dodo, Hortense W. (Department of Food and Animal Sciences, School of Agricultural and Environmental Sciences, Alabama A+M University, Normal, AL, USA). Applied Biotechnology, Food Science and Policy, 1(3), 159-168 (English) 2003. CODEN: ABFSBL. ISSN: 1175-9534. Publisher: Open Mind Journals.

AB Peanut allergy is a severe, persistent and life-threatening food allergy. Ara h 2 is the most offending allergen in peanut, recognized by the serum IgE of more than 90% of peanut-allergic individuals. Genetic manipulation of peanut can be used as an approach to render this nutritive crop safer for human consumption. The objective of this research was to determine the expression of a truncated fragment from the coding region of the allergen Ara h 2 in transgenic peanut, which is a prerequisite for silencing this allergen. Peanut was transformed with a 430-bp truncated Ara h 2 gene, inserted between an enhanced CaMV 35S promoter and the Nos terminator. This construct, named pDK2, was used in co-transformation with either plasmid pB1426 containing the NPTII selection marker gene, or plasmid pCB13 containing the hygromycin selection marker gene. Southern blot analyses showed the stable integration of the transgene into the peanut genome, and Northern blot analyses detected the transgene transcripts in the calluses, leaves and roots, confirming the constitutive expression of these transcripts throughout the development of the transgenic plants. These results indicate that the truncated Ara h 2 transgene fulfilled all the requirements to be expressed in the transgenic seeds and trigger the down-regulation of the Ara h 2 gene, thus leading to a decrease in peanut allergenicity.

L26 ANSWER 13 OF 32 EMBASE

on STN
2003223192 EMBASE Isolation and characterization of two complete Ara h 2 isoforms cDNA. Chatel J.-M.; Bernard H.; Orson F.M.. Dr. J.-M. Chatel, INRA-Lab. Immuno-Allergie A., CE Saclay, Bat. 136, F-91191 Gif-sur-Yvette, United States. chatel@dsvidf.cea.fr. International Archives of Allergy and Immunology Vol. 131, No. 1, pp. 14-18 2003.
Refs: 16.
ISSN: 1018-2438. CODEN: IAAIEG
Pub. Country: Switzerland. Language: English. Summary Language: English.
ED Entered STN: 20030619
AB Background: Ara h 2 is a major peanut allergen recognized by IgE in more than 90% of patients. After electrophoretic separation the purified protein exists as a doublet, and sequences of one incomplete cDNA and one genomic clone for this allergen have been reported. Methods: Ara h 2 isoforms were purified and analyzed by mass spectroscopy, and PCR amplification products of Ara h 2 were cloned and sequenced. Results: Mass spectroscopy of purified Ara h 2 clearly identified a molecular doublet of 16,670 and 18,050 Daltons. Amplification of a peanut cDNA library using PCR primer pairs located at the amino- and carboxy-terminus revealed 2 bands separated by 50 base pairs, which we cloned and sequenced. Two types of complete cDNA clones were obtained, Ara h 2.01 and Ara h 2.02. Compared to Ara h 2.01 and the previously reported cDNA sequences, Ara h 2.02 is characterized by a 12 amino acid insertion starting at position 75 that contains a third repeat of the major IgE binding epitope DPYSPS. Conclusion: We demonstrated the molecular and genetic characteristics of two Ara h 2 isoforms, revealing that one, Ara h 2.02, might be the more potent allergen. Copyright .COPYRGT. 2003 S. Karger AG, Basel.

L26 ANSWER 14 OF 32 CAPLUS COPYRIGHT 2005 ACS on STN
2002:186962 Extent of glycosylation and resistance to digestion of allergenic proteins isolated from a single peanut. Spivey, Margaret; Kopper, Randall A. (Department of Chemistry, Hendrix College, Conway, AR, 72032, USA). Abstracts of Papers, 223rd ACS National Meeting, Orlando, FL, United States, April 7-11, 2002, CHED-331. American Chemical Society: Washington, D. C. (English) 2002. CODEN: 69CKQP.
AB Peanut allergy is a serious hypersensitivity reaction to one or more peanut protein allergens. Among these allergenic proteins, Ara h 2 is one of the most important. It exists as two isoforms with the same amino acid sequence, but different degrees of glycosylation. This study involves a comparison of the relative amts. of the two electrophoretically distinct Ara h 2 isoforms found in protein prepns. from single peanuts. Microscale methods are being developed for the purification of Ara h 2 from a single peanut. Gel electrophoresis of the resulting proteins will indicate how glycosylation patterns vary in peanuts produced from different plants. In order to assess the importance of glycosylation of the allergen to the action of digestive proteases, **Ara h2** will be exposed to enzymes such as trypsin and the stability of the protein assessed by gel electrophoresis. These results will provide a link between allergen glycosylation and the resistance of the protein to digestive enzymes.

L26 ANSWER 15 OF 32 CAPLUS COPYRIGHT 2005 ACS on STN
2002:186951 Effect of protein structure on the digestion and allergenicity of a major peanut allergen. Williams, Meghan H.; Kopper, Randall (Department of Chemistry, Hendrix College, Conway, AR, 72032, USA). Abstracts of Papers, 223rd ACS National Meeting, Orlando, FL, United States, April 7-11, 2002, CHED-320. American Chemical Society: Washington, D. C. (English) 2002. CODEN: 69CKQP.
AB Hypersensitivity to peanuts is a reaction in response to one or more peanut protein allergens. Ara h 2 is one of the most commonly recognized allergenic proteins. The importance of secondary and tertiary structure to the overall stability of the allergen toward the action of digestive proteases was investigated. **Ara h2** was exposed to pepsin, trypsin or chymotrypsin and the stability of the protein was assessed by gel electrophoresis. Upon enzyme treatment, a number of

relatively large protein fragments are produced which are resistant to further enzymic digestion. The enzyme-treated allergen remains essentially intact despite the action of proteases until the fragments are dissociated when the disulfide linkages are reduced. A highly resistant 10 kDa peptide contains intact IgE-binding epitopes and several potential enzyme cut sites that are protected from the enzymes by the compact structure of the protein. These results provide a link between allergen structure and the preservation of IgE-binding epitopes during digestion.

L26 ANSWER 16 OF 32 BIOSIS COPYRIGHT (c) 2005 The Thomson Corporation on STN DUPLICATE 2

2002:301849 Document No.: PREV200200301849. Relative potency of the major peanut allergens, Ara h1 and **Ara h2**, in a functional assay. Palmer, G. William [Reprint author]; Dibbern, Donald A. [Reprint author]; Burks, Wesley; Bannon, Gary A.; Bock, S. Allan; Dreskin, Stephen C. [Reprint author]. University of Colorado, Denver, CO, USA. Journal of Allergy and Clinical Immunology, (January, 2002) Vol. 109, No. 1 Supplement, pp. S300. print.
Meeting Info.: 58th Annual Meeting of the American Academy of Allergy, Asthma and Immunology. New York, NY, USA. March 01-06, 2002. American Academy of Allergy, Asthma, and Immunology.
CODEN: JACIBY. ISSN: 0091-6749. Language: English.

L26 ANSWER 17 OF 32 SCISEARCH COPYRIGHT (c) 2005 The Thomson Corporation on STN

2002:203179 The Genuine Article (R) Number: 519UX. Protein structure plays a critical role in peanut allergen **Ara h2** stability and may determine immunodominant IgE binding epitopes. Sen M (Reprint); Kopper R; Pons L; Abraham E C; Burks W; Bannon G A. Arkansas Childrens Hosp, Res Inst, Little Rock, AR 72202 USA; Univ Arkansas, Little Rock, AR 72204 USA. JOURNAL OF ALLERGY AND CLINICAL IMMUNOLOGY (JAN 2002) Vol. 109, No. 1, Supp. [S], pp. S300-S300. MA 919. Publisher: MOSBY, INC. 11830 WESTLINE INDUSTRIAL DR, ST LOUIS, MO 63146-3318 USA. ISSN: 0091-6749. Pub. country: USA. Language: English.

L26 ANSWER 18 OF 32 BIOSIS COPYRIGHT (c) 2005 The Thomson Corporation on STN

2002:301398 Document No.: PREV200200301398. Immunotherapy for peanut allergy using modified allergens and a bacterial adjuvant. Stanley, Joseph Steve [Reprint author]; Buzen, Fred [Reprint author]; Cockrell, Gael [Reprint author]; West, Mike [Reprint author]; Srivastava, Kamal D.; Li, X. M.; Sampson, Hugh A.; Burks, Wesley [Reprint author]; Bannon, Gary A. [Reprint author]. University of Arkansas, Little Rock, AR, USA. Journal of Allergy and Clinical Immunology, (January, 2002) Vol. 109, No. 1 Supplement, pp. S93. print.
Meeting Info.: 58th Annual Meeting of the American Academy of Allergy, Asthma and Immunology. New York, NY, USA. March 01-06, 2002. American Academy of Allergy, Asthma, and Immunology.
CODEN: JACIBY. ISSN: 0091-6749. Language: English.

L26 ANSWER 19 OF 32 CAPLUS COPYRIGHT 2005 ACS on STN

2002:940522 Document No. 138:336553 Detection methods for allergic substances in foods by enzyme linked immunosorbent assay. Honjoh, Tsutomu; Muraoka, Shiroo; Mamegoshi, Shinichi; Sakai, Masatoshi (Morinaga Inst. Biological Sci., Tsurumi-ku, Yokohama-shi, Kanagawa, 230-8504, Japan). Foods & Food Ingredients Journal of Japan, 206, 13-22 (Japanese) 2002. CODEN: FFIJER. ISSN: 0919-9772. Publisher: FFI Janaru.

AB A review. Highly sensitive ELISA systems that can measure proteins in egg, milk, wheat, buckwheat and peanut have been established. Ovalbumin and ovomucoid were selected as the target proteins to detect egg, casein and beta-lactoglobulin, to detect milk and gliadin, to detect wheat major protein complex, to detect buckwheat and protein complex that contains **Ara h2**, and to detect peanut. The detection limit of these ELISA systems are 1 ng/mL total protein of each material in the exts. Cross reactivity is rarely observed in these systems, however rye extract

strongly reacts with the wheat system, smartweed reacts with the buckwheat system and chicken meat reacts with the ovomucoid system. These cross reactions are confirmed by ELISA systems using monoclonal antibodies to each material, western blot anal. that uses monoclonal and polyclonal antibodies to each material and PCR reactions. Sensitivity of established western blot systems are 0.3 ppm for ovalbumin, and 1.3 ppm for beta-lactoglobulin, casein, and ovomucoid in the samples, resp. The results of spike and recovery techniques are satisfactory. These systems are widely applicable to egg, milk, wheat, buckwheat and peanut. But these systems cannot detect allergens in canned foods and bottled foods, which are treated at high temperature under high-pressure conditions. These systems have been established as kits, which are very easy to use. The ELISA system and western blot anal. are powerful detection methods for foods which contain egg, milk, wheat, buckwheat and peanut.

L26 ANSWER 20 OF 32 CAPLUS COPYRIGHT 2005 ACS on STN
2001:380762 Document No. 135:1229 Isolation and sequence of a full length genomic clone for allergen **Ara h2** and down-regulation and silencing of allergen genes in transgenic peanut seeds. Dodo, Hortense W.; Arntzen, Charles J.; Konan, Koffi N'da; Viquez, Olga M. (Alabama A + M University, USA). PCT Int. Appl. WO 2001036621 A2 20010525, 73 pp. DESIGNATED STATES: W: AE, AG, AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, BZ, CA, CH, CN, CR, CU, CZ, DE, DK, DM, DZ, EE, ES, FI, GB, GD, GE, GH, GM, HR, HU, ID, IL, IN, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MA, MD, MG, MK, MN, MW, MX, MZ, NO, NZ, PL, PT, RO, RU, SD, SE, SG, SI, SK, SL, TJ, TM, TR, TT, TZ, UA, UG, US, UZ, VN, YU, ZA, ZW, AM, AZ, BY, KG, KZ, MD, RU, TJ, TM; RW: AT, BE, BF, BJ, CF, CG, CH, CI, CM, CY, DE, DK, ES, FI, FR, GA, GB, GR, IE, IT, LU, MC, ML, MR, NE, NL, PT, SE, SN, TD, TG, TR. (English). CODEN: PIXXD2. APPLICATION: WO 2000-US31657 20001120. PRIORITY: US 1999-PV167255 19991119.

AB An allergen-free transgenic peanut seed is produced by recombinant methods. Peanut plants are transformed with multiple copies of each of the allergen genes, or fragments thereof, to suppress gene expression and allergen protein production Alternatively, peanut plants are transformed with peanut allergen antisense genes introduced into the peanut genome as antisense fragments, sense fragments, or combinations of both antisense and sense fragments. Peanut transgenes are under the control of the 35S promoter, or the promoter of the **Ara h2** gene to produce antisense RNAs, sense RNAs, and double-stranded RNAs for suppressing allergen protein production in peanut plants. A full length genomic clone for allergen **Ara h2** is isolated and sequenced. The ORF is 622 nucleotides long. The predicted encoded protein is 207 amino acids long and includes a putative transit peptide of 21 residues. One polyadenilation signal is identified at position 951. Six addnl. stop codons are observed A promoter region was revealed containing a putative TATA box located at position -72. Homologous regions were identified between **Ara h2**, h6, and h7, and between Ara h3 and h4, and between Ara h1P41B and Ara h1P17. The homologous regions will be used for the screening of peanut genomic library to isolate all peanut allergen genes and for down-regulation and silencing of multiple peanut allergen genes.

L26 ANSWER 21 OF 32 MEDLINE on STN DUPLICATE 3
2001248467. PubMed ID: 11274350. A strategy for the identification of proteins targeted by thioredoxin. Yano H; Wong J H; Lee Y M; Cho M J; Buchanan B B. (Department of Plant and Microbial Biology, University of California, Berkeley, CA 94720, USA.) Proceedings of the National Academy of Sciences of the United States of America, (2001 Apr 10) 98 (8) 4794-9. Electronic Publication: 2001-03-27. Journal code: 7505876. ISSN: 0027-8424. Pub. country: United States. Language: English.

AB Thioredoxins are 12-kDa proteins functional in the regulation of cellular processes throughout the animal, plant, and microbial kingdoms. Growing evidence with seeds suggests that an h-type of thioredoxin, reduced by

NADPH via NADP-thioredoxin reductase, reduces disulfide bonds of target proteins and thereby acts as a wakeup call in germination. A better understanding of the role of thioredoxin in seeds as well as other systems could be achieved if more were known about the target proteins. To this end, we have devised a strategy for the comprehensive identification of proteins targeted by thioredoxin. Tissue extracts incubated with reduced thioredoxin are treated with a fluorescent probe (monobromobimane) to label sulfhydryl groups. The newly labeled proteins are isolated by conventional two-dimensional electrophoresis: (i) nonreducing/reducing or (ii) isoelectric focusing/reducing SDS/PAGE. The isolated proteins are identified by amino acid sequencing. Each electrophoresis system offers an advantage: the first method reveals the specificity of thioredoxin in the reduction of intramolecular vs. intermolecular disulfide bonds, whereas the second method improves the separation of the labeled proteins. By application of both methods to peanut seed extracts, we isolated at least 20 thioredoxin targets and identified 5-three allergens (**Ara h2**, Ara h3, and Ara h6) and two proteins not known to occur in peanut (desiccation-related and seed maturation protein). These findings open the door to the identification of proteins targeted by thioredoxin in a wide range of systems, thereby enhancing our understanding of its function and extending its technological and medical applications.

L26 ANSWER 22 OF 32 CAPLUS COPYRIGHT 2005 ACS on STN

2001:295491 Document No. 135:151859 Detection of peanut allergens in breast milk of lactating women. Vadas, Peter; Wai, Yvonne; Burks, Wesley; Perelman, Boris (Division of Allergy and Clinical Immunology, St Michael's Hospital, University of Toronto, Toronto, ON, Can.). JAMA, the Journal of the American Medical Association, 285(13), 1746-1748 (English) 2001. CODEN: JAMAAP. ISSN: 0098-7484. Publisher: American Medical Association.

AB Most individuals who react to peanuts do so on their 1st known exposure. A potential but unproven route of occult exposure resulting in sensitization to peanut is via breast milk during lactation. To investigate the ability of maternal dietary peanut protein to pass into breast milk during lactation. Clin. investigation conducted at 2 North American hospitals from Mar. 1999 to Oct. 2000 including 23 healthy, lactating women aged 21-35 yr. Each woman consumed 50 g of dry roasted peanuts, after which breast milk samples were collected at hourly intervals. Presence in breast milk of total peanut protein, analyzed by a sandwich ELISA, and 2 major peanut allergens, Ara h 1 and Ara h 2, detected by immunoblot anal. Peanut protein was detected in 11 of 23 subjects. It was detected in 10 subjects within 2 h of ingestion and in 1 subject within 6 h. The median peak peanut protein concentration in breast milk was 200 ng/mL (mean, 222 ng/mL; range, 120-430 ng/mL). Both major peanut allergens Ara h 1 and Ara h 2 were detected. Conclusions Peanut protein is secreted into breast milk of lactating women following maternal dietary ingestion. Exposure to peanut protein during breastfeeding is a route of occult exposure that may result in sensitization of at-risk infants.

L26 ANSWER 23 OF 32 CAPLUS COPYRIGHT 2005 ACS on STN

2001:493625 Document No. 135:287805 Effects of cooking methods on peanut allergenicity. Beyer, Kirsten; Morrow, Ellen; Li, Xiu-Min; Bardina, Ludmilla; Bannon, Gary A.; Burks, A. Wesley; Sampson, Hugh A. (Division of Pediatric Allergy & Immunology and Jaffe Institute for Food Allergy, The Mount Sinai School of Medicine, New York, NY, 10029-6574, USA). Journal of Allergy and Clinical Immunology, 107(6), 1077-1081 (English) 2001. CODEN: JACIBY. ISSN: 0091-6749. Publisher: Mosby, Inc..

AB Allergy to peanut is a significant health problem. Interestingly, the prevalence of peanut allergy in China is much lower than that in the United States, despite a high rate of peanut consumption in China. In China, peanuts are commonly fried or boiled, whereas in the United States peanuts are typically dry roasted. The aim of this study was to examine whether the method of preparing peanuts could be a factor in the disparity of allergy prevalence between the 2 countries. Two varieties of peanuts grown in the United States were roasted, boiled, or fried. Proteins were

analyzed by using SDS-PAGE and immunoblotting. Allergenicity was compared by using immunolabeling with sera from 8 patients with peanut allergy. The protein fractions of both varieties of peanuts were altered to a similar degree by frying or boiling. Compared with roasted peanuts, the relative amount of Ara h 1 was reduced in the fried and boiled prepns., resulting in a significant reduction of IgE-binding intensity. In addition, there was significantly less IgE binding to Ara h 2 and Ara h 3 in fried and boiled peanuts compared with that in roasted peanuts, even though the protein amts. were similar in all 3 prepns. The methods of frying or boiling peanuts, as practiced in China, appear to reduce the allergenicity of peanuts compared with the method of dry roasting practiced widely in the United States. Roasting uses higher temps. that apparently increase the allergenic property of peanut proteins and may help explain the difference in prevalence of peanut allergy observed in the 2 countries.

L26 ANSWER 24 OF 32 CAPLUS COPYRIGHT 2005 ACS on STN
2001:363205 Document No. 136:101378 Isolation and molecular characterization of the first genomic clone of a major peanut allergen, Ara h 2. Viquez, Olga M.; Summer, Cathrine G.; Dodo, Hortense W. (Department of Food and Animal Sciences, Alabama A and M University, Normal, AL, 35762, USA). Journal of Allergy and Clinical Immunology, 107(4), 713-717 (English) 2001. CODEN: JACIBY. ISSN: 0091-6749. Publisher: Mosby, Inc..
AB Peanuts have been identified as potent food allergens responsible for life-threatening IgE reactions among hypersensitive individuals. With the current increase of peanut allergies, there is an urgent need to molecularly characterize the genes encoding the target proteins and to understand the nature of their regulation. The objectives of this study were to isolate, sequence, and characterize at least one full-length genomic clone encoding the major peanut allergen Ara h 2. A peanut genomic library, constructed in a Lambda Fix II vector, was screened with an 80-bp oligonucleotide probe constructed on the basis of the 5' end of a published Ara h 2 cDNA partial sequence. One putative pos. lambda clone was isolated, digested with BamHI to release its 16-kb insert, and confirmed by dot blot and Southern hybridization. The pos. clone was subcloned in pBluescript SK+ vector, sequenced, and characterized. Sequence anal. revealed a full-length genomic clone with an open reading frame starting with an initiation codon (ATG) at position 1 and ending with a termination codon (TGA) at position 622. One putative polyadenylation signal (AATAAA) is identified at positions 951 in the 3' untranslated region, and 6 addnl. stop codons are located at positions 628, 769, 901, 946, 967, and 982 downstream from the start codon. In the 5' promoter region, a putative TATA box (TATTATTA) is located at position -72 upstream from the start codon. The deduced amino acid sequence has 207 residues and includes a putative signal peptide of 21 residues. The results reveal for the first time information on the structure of a major peanut allergen, Ara h 2. Comparison of the cDNA and genomic sequences revealed the absence of an intron but the presence of 2 isoforms of Ara h 2 or different members of the same gene family.

L26 ANSWER 25 OF 32 BIOSIS COPYRIGHT (c) 2005 The Thomson Corporation on STN DUPLICATE 4
2001:187787 Document No.: PREV200100187787. Investigation of the use of ISS-linked **Ara h2** for the treatment of peanut-induced allergy. Srivastava, K. [Reprint author]; Li, X.-M.; Bannon, G. A.; Burks, A. W.; Eiden, J.; Vannest, G.; Tuck, R.; Rodriguez, R.; Sampson, H. A.. Mount Sinai School of Medicine, New York, NY, USA. Journal of Allergy and Clinical Immunology, (February, 2001) Vol. 107, No. 2, pp. S233. print. Meeting Info.: 57th Annual Meeting of the American Academy of Allergy, Asthma and Immunology. New Orleans, Louisiana, USA. March 16-21, 2001. American Academy of Allergy Asthma and Immunology.
CODEN: JACIBY. ISSN: 0091-6749. Language: English.

L26 ANSWER 26 OF 32 BIOSIS COPYRIGHT (c) 2005 The Thomson Corporation on STN
2001:178088 Document No.: PREV200100178088. Role of conformational and

linearized epitopes in the achievement of tolerance in peanut allergy. Ellman, Lisa Kim [Reprint author]; Beyer, Kirsten [Reprint author]; Bardina, Ludmilla [Reprint author]; Jarvinen, Kirsi-Marjut [Reprint author]; Bannon, Gary A.; Burks, Wesley; Sampson, Hugh A. [Reprint author]. Mount Sinai School of Medicine, New York, NY, USA. Journal of Allergy and Clinical Immunology, (February, 2001) Vol. 107, No. 2, pp. S139. print.
Meeting Info.: 57th Annual Meeting of the American Academy of Allergy, Asthma and Immunology. New Orleans, Louisiana, USA. March 16-21, 2001. American Academy of Allergy Asthma and Immunology.
CODEN: JACIBY. ISSN: 0091-6749. Language: English.

L26 ANSWER 27 OF 32 MEDLINE on STN DUPLICATE 5
2001322186. PubMed ID: 11167950. Polyisotypic antipeanut-specific humoral responses in peanut-allergic individuals. Kolopp-Sarda M N; Moneret-Vautrin D A; Gobert B; Kanny G; Guerin L; Faure G C; Bene M C. (Laboratoire d'Immunologie, Faculte de Medecine & CHU de Nancy, 54500 Vandoeuvre-les-Nancy, France.) Clinical and experimental allergy : journal of the British Society for Allergy and Clinical Immunology, (2001 Jan) 31 (1) 47-53. Journal code: 8906443. ISSN: 0954-7894. Pub. country: England: United Kingdom. Language: English.
AB BACKGROUND: Peanut-containing food products may induce severe clinical reactions in sensitized subjects, and high levels of antipeanut IgE have been reported in the literature. Immunotherapy, proposed for the prevention of severe accidents, is often ill-tolerated and only partly efficient. This could be due to the spontaneous development of polyisotypic antipeanut antibodies. OBJECTIVE: To appreciate the presence and reactivity of other isotypes other than IgE of peanut-specific antibodies in serum samples from peanut-sensitized subjects. METHODS: Serum samples were obtained from 20 non-sensitized subjects and 23 sensitized patients divided in three groups according to their response to peanut oral challenge (no response or response to high or low doses, respectively). Peanut-specific IgG, IgG subclasses, IgA and IgM were assayed using an ELISA, and their reactivity against peanut proteins tested using Western Blot. RESULTS: A large dispersion of antipeanut antibody levels was observed in the three groups of patients, high levels of IgG, IgG1, IgG4 and IgA usually correlating with highly positive radioallergosorbent test (RAST). Such high levels were observed at onset in four patients who underwent peanut immunotherapy who had side effects and poor efficiency. Western blotting demonstrated that the polyisotypic response observed was directed to several peanut antigens, including the major allergens, Ara h1 and **Ara h2.** CONCLUSION:
Peanut-sensitized patients who spontaneously develop specific IgE, display polyisotypic-specific antibody responses, whatever their response to oral challenge. This might explain the poor efficiency of peanut rush immunotherapy attempts.

L26 ANSWER 28 OF 32 CAPLUS COPYRIGHT 2005 ACS on STN
2002:277584 Document No. 136:368007 Peanut allergy: From allergens to immunotherapy. Helm, Ricki M.; Sampson, Hugh A.; Bannon, Gary A.; Burks, A. Wesley (Arkansas Children's Hospital, University of Arkansas for Medical Sciences, Little Rock, AR, 72202, USA). Recent Research Developments in Allergy & Clinical Immunology, 1, 11-21 (English) 2000.
CODEN: RRDACJ. Publisher: Research Signpost.
AB A review. Peanut hypersensitivity represents a significant health problem to the peanut sensitive population because of the potential severity, life-long nature, and ubiquitous use of peanut products. The relevant allergens have been identified using serum IgE from peanut-sensitive individuals using physicochem. and immunol. procedures. Ara h 1 and 2 represent major allergens that bind IgE from greater than 90% of the sensitive population. Ara h 3 and other peanut allergens bind serum IgE from less than 50% of the sensitive population. Major linear IgE-binding epitopes have been identified for each of the allergens using recombinant proteins from a cDNA expression library. Mutational anal. of epitopes by alanine substitution indicates that a single amino acid change results in

loss of IgE binding. Ara h 1 and 2 are being used to investigate the potential application of vaccine development in the modulation of food allergy. Results from these investigations will allow for the design of new diagnostic and immunotherapeutic approaches to peanut hypersensitivity.

L26 ANSWER 29 OF 32 MEDLINE on STN DUPLICATE 6
1999172246. PubMed ID: 10072557. Strain-dependent induction of allergic sensitization caused by peanut allergen DNA immunization in mice. Li X; Huang C K; Schofield B H; Burks A W; Bannon G A; Kim K H; Huang S K; Sampson H A. (Department of Pediatrics, Mount Sinai School of Medicine, New York, NY 10029, USA.. Xiu-min_li@smtplink.mssm.edu) . Journal of immunology (Baltimore, Md. : 1950), (1999 Mar 1) 162 (5) 3045-52. Journal code: 2985117R. ISSN: 0022-1767. Pub. country: United States. Language: English.

AB To investigate the potential application of allergen gene immunization in the modulation of food allergy, C3H/HeSn (C3H) mice received i.m. injections of pAra h2 plasmid DNA encoding one of the major peanut allergens, **Ara h2**. Three weeks following pDNA immunization, serum **Ara h2**-specific IgG2a, IgG1, but not IgE, were increased significantly in a dose-dependent manner. IgG1 was 30-fold higher in multiply compared with singly immunized mice. **Ara h2** or peanut protein injection of immunized mice induced anaphylactic reactions, which were more severe in multiply immunized mice. Heat-inactivated immune serum induced passive cutaneous anaphylaxis, suggesting that anaphylaxis in C3H mice was mediated by IgG1. IgG1 responses were also induced by intradermal injection of pAra h2, and by i.m. injection of pOMC, the plasmid DNA encoding the major egg allergen protein, ovomucoid. To elucidate whether the pDNA immunization-induced anaphylaxis was a strain-dependent phenomenon, AKR/J and BALB/c mice also received multiple i.m. pAra h2 immunizations. Injection of peanut protein into these strains at weeks 3 or 5 following immunization did not induce reactions. Although IgG2a was increased significantly from week 2 in AKR/J mice and from week 4 in BALB/c mice and remained elevated for at least 6 wk, no IgG1 or IgE was detected. These results indicate that the type of immune responses to pDNA immunization in mice is strain dependent. Consequently, models for studying human allergen gene immunization require careful selection of suitable strains. In addition, this suggests that similar interindividual variation is likely in humans.

L26 ANSWER 30 OF 32 MEDLINE on STN DUPLICATE 7
1998339794. PubMed ID: 9677140. Identification and partial characterization of multiple major allergens in peanut proteins. de Jong E C; Van Zijverden M; Spanhaak S; Koppelman S J; Pellegrom H; Penninks A H. (TNO Nutrition and Food Research Institute, Immunotoxicology group, Zeist, The Netherlands.) Clinical and experimental allergy : journal of the British Society for Allergy and Clinical Immunology, (1998 Jun) 28 (6) 743-51. Journal code: 8906443. ISSN: 0954-7894. Pub. country: ENGLAND: United Kingdom. Language: English.

AB BACKGROUND: Peanuts are a major cause of food allergies both in children as in adults which can induce an anaphylactic shock. The identification and characterization of peanut allergens could lead to more insight into the mechanism and contribute to the improvement of diagnostic tests and treatment for peanut allergy. OBJECTIVE: In the present study, the peanut protein-specific immunoglobulin concentrations as well as their recognition of the various peanut proteins or protein subunits was determined in the plasma of peanut-allergic (PA) and non-allergic (NA) individuals. Moreover, two peanut allergens were characterized in more detail to confirm them as the earlier described Ara h1 and **Ara h2**. METHODS: The presence of Ig-binding sites in peanut proteins was studied by immunoblotting assays whereas the concentrations of peanut-specific Ig was determined by ELISA. RESULTS: Peanut proteins were found to contain multiple binding sites for immunoglobulins. Of these proteins, six were recognized by peanut-specific IgE present in more than 50% of the plasma samples of the PA group. Their molecular weights were

approximately 44, 40, 33, 21, 20 and 18 kDa. The last three protein bands were recognized by peanut-specific IgE present in more than 70% of the PA plasma samples and were thought to contain **Ara h2.** This allergen as well as another protein that was thought to be Ara h1, which was not recognized by the majority of the patients' IgE-containing plasma samples, were isolated and the N terminal amino acid sequence was determined. Peanut protein-specific IgA, IgM, IgG and IgG-subclasses showed a more diverse recognition pattern of peanut protein in the PA group compared to the NA group. No differences were found in the plasma concentrations of peanut protein-specific immunoglobulins of the various classes between the PA and NA group. CONCLUSIONS: From the present study, we conclude that peanuts contain multiple allergens, of which six can be described as major allergens, **Ara h2** included. In our population Ara h1 is not a major allergen. The recognition of peanut proteins by immunoglobulins is more diverse in PA individuals compared with NA individuals which, however, is not substantiated in the concentrations of peanut-specific immunoglobulins in plasma, other than IgE.

L26 ANSWER 31 OF 32 CAPLUS COPYRIGHT 2005 ACS on STN

1996:612305 Document No. 125:273253 Stability of food allergens to digestion in vitro. Astwood, James D.; Leach, John N.; Fuchs, Roy L. (Monsanto Co., St Louis, MO, 63198, USA). Nature Biotechnology, 14(10), 1269-1273 (English) 1996. CODEN: NABIF9. ISSN: 1087-0156. Publisher: Nature Publishing Co..

AB An integral part of the safety assessment of genetically modified plants is consideration of possible human health effects, especially food allergy. Prospective testing for allergenicity of proteins obtained from sources with no prior history of causing allergy has been difficult because of the absence of valid methods and models. Food allergens may share physicochem. properties that distinguish them from nonallergens, properties that may be used as a tool to predict the inherent allergenicity of proteins newly introduced into the food supply by genetic engineering. One candidate property is stability to digestion. We have systematically evaluated the stability of food allergens that are active via the gastrointestinal tract in a simple model of gastric digestion, emphasizing the major allergens of plant-derived foods such as legumes (peanuts and soybean). Important food allergens were stable to digestion in the gastric model (simulated gastric fluid). For example, soybean β-conglycinin was stable for 60 min. In contrast, nonallergenic food proteins, such as spinach ribulose bis-phosphate carboxylase/oxygenase, were digested in simulated gastric fluid within 15 s. The data are consistent with the hypothesis that food allergens must exhibit sufficient gastric stability to reach the intestinal mucosa where absorption and sensitization (development of atopy) can occur. Thus, the stability to digestion is a significant and valid parameter that distinguishes food allergens from nonallergens.

L26 ANSWER 32 OF 32 BIOSIS COPYRIGHT (c) 2005 The Thomson Corporation on STN DUPLICATE 8

1986:454896 Document No.: PREV198682111738; BA82:111738. HYDROGEN EVOLUTION AND RELATIVE EFFICIENCY IN CHICK PEA CICER-ARIETINUM EFFECT OF RHIZOBIUM STRAIN HOST CULTIVAR AND TEMPERATURE. SINDHU S S [Reprint author]; DADARWAL K R; DAHIYA B S. DEP OF MICROBIOL, HARYANA AGRICULTURAL UNIV, HISSAR 125 004, INDIA. Indian Journal of Experimental Biology, (1986) Vol. 24, No. 7, pp. 416-420.
CODEN: IJEBA6. ISSN: 0019-5189. Language: ENGLISH.

AB Effect of Rhizobium strains, host cultivars, plant age and temperature on acetylene reduction activity (**ARA**), **H2** evolution and relative efficiency (RE) were studied in C. arietinum. The Rhizobium strains nodulating this legume were observed to be of Hup- phenotype. At soil temperature prevailing during growth and active nitrogen fixation stage an average RE of 0.81 was observed in cultivar C235 up to day 90 of plant growth by inoculating different Hup- strains. Variance due to Rhizobium strains in RE was not significant. Relative ARA values were

initially low at day 45 of plant growth which increased at day 60 followed by a slight decline at day 90. However, no significant differences in RE values were observed due to plant age except in case of those strains where early degeneration of nodules started, resulting into decrease in RE. Also, Rhizobium strains differing in effectivity, showing difference in plant dry weight ratios from less than 3.0 to more than 8.0, did not differ significantly in RE. Host cultivars did not change significantly RE of different Rhizobium strains. Effects of temperature on ARA and H2 evolution were studied in eight cultivars grown under field conditions and nodulated with native Rhizobium strains. The average RE when measured at 20° C, in these cultivars was above 0.8 from day 60 to 105 of plant growth. Hydrogen uptake activity was absent in nodules of all cultivars. At 25° C, RE decreased in all cultivars, mainly due to decrease in relative ARA. Cultivar effect on RE was more pronounced at 25° C as two of the cultivars H82-2 and H75-35 showed significantly low RE at this temperature. H2 evolution in C2H2 atmosphere was increased 2-fold at 25° C as compared to 20° C in nodules of all the cultivars.

=> s Ara h3
L27 39 ARA H3

=> dup remove l27
PROCESSING COMPLETED FOR L27
L28 30 DUP REMOVE L27 (9 DUPLICATES REMOVED)

=> d l28 1-30 cbib abs

L28 ANSWER 1 OF 30 MEDLINE on STN
2005181166. PubMed ID: 15813812. Peanut- and cow's milk-specific IgE, Th2 cells and local anaphylactic reaction are induced in Balb/c mice orally sensitized with cholera toxin. Adel-Patient K; Bernard H; Ah-Leung S; Creminon C; Wal J-M. (Laboratoire Inra d'Immuno-Allergie Alimentaire, CEA de Saclay, Gif sur Yvette cedex-France.) Allergy, (2005 May) 60 (5) 658-64. Journal code: 7804028. ISSN: 0105-4538. Pub. country: Denmark. Language: English.

AB Background: The development of animal models developing specific immunoglobulin (Ig)E presenting the same specificity as human IgE and similar clinical symptoms as those observed in allergic patients are of great interest for the understanding of mechanisms involved in the induction and regulation of food allergy. Methods: Balb/c female mice were sensitized with whole peanut protein extract (WPPE) by means of intraperitoneal (i.p.) injections with alum or gavages with cholera toxin (CT). The WPPE specific IgE, IgG1 and IgG2a were monitored. Th2 cells activation was analysed assaying interleukin (IL)-4 and IL-5 vs IFNgamma on reactivated splenocytes. Local anaphylactic reaction was evaluated by assaying histamine in faecal samples. The oral sensitization protocol was further extended to cow's milk proteins (CMP). Results: Balb/c mice developed high peanut-specific IgE and IgG1 responses either after i.p. or oral sensitizations. In both cases, antibodies were specific to polymer of glycinin fragments, containing polypeptides from **Ara h3**/4, and to a lesser extent to Ara h1 and Ara h2. Interleukin-4 and IL-5 production were evidenced. Balb/c mice could also be sensitized to CMP, as demonstrated by CMP-specific IL-4 and IL-5 secretions and induction of IgE specific for whole caseins, beta-lactoglobulin, serum bovine albumin and lactoferrin. Of interest was the occurrence of a local anaphylactic reaction in the peanut and CM models. Conclusions: In contrast with previous authors, Balb/c mice were sensitized and evidenced an allergic reaction after oral administrations of peanut or CMP plus CT, providing an interesting model for further studies on immunopathogenic mechanisms.

L28 ANSWER 2 OF 30 MEDLINE on STN
2005134330. PubMed ID: 15765743. Identification of the basic subunit of

Ara h 3 as the major allergen in a group of children allergic to peanuts. Restani Patrizia; Ballabio Cinzia; Corsini Emanuela; Fiocchi Alessandro; Isoardi Patrizia; Magni Chiara; Poiesi Claudio; Terracciano Luigi; Duranti Marcello. (Department of Pharmacological Sciences, University of Milano, Milano, Italy.. patrizia.restani@unimi.it) . Annals of allergy, asthma & immunology : official publication of the American College of Allergy, Asthma, & Immunology, (2005 Feb) 94 (2) 262-6. Journal code: 9503580. ISSN: 1081-1206. Pub. country: United States. Language: English.

AB BACKGROUND: Several proteins have been identified as peanut allergens; among them, Ara h 1 (7S globulin) and Ara h 2 (2S globulin) are usually considered the major allergens. OBJECTIVE: To identify the major allergens in a group of children selected for their specific pattern of immunoreactivity. METHODS: We identified the dominant allergen by using (1) amino acid sequencing of the bands that show the strongest IgE immunoreactivity in 1-dimensional electrophoresis and immunoblotting and (2) specific animal IgGs raised against the dominant immunoreactive band to pinpoint the allergen(s) in peanut proteins separated by 2-dimensional electrophoresis and immunoblotting. To confirm these data, we further examined the peanut proteome using serum samples from the children with the unusual immunoreactivity. RESULTS: We found a group of children with marked peanut allergy who are specifically sensitized to the basic subunit of Ara h 3 (11S globulin family). CONCLUSION: That the dominant immunoreactivity in these patients is in a basic subunit of Ara h 3 was unexpected, because previous studies had indicated that Ara h 3 was only a minor peanut allergen and that the identified allergenic epitopes occurred mainly in the acidic Ara h 3 subunit.

L28 ANSWER 3 OF 30 CAPLUS COPYRIGHT 2005 ACS on STN

2004:252189 Document No. 140:286142 Hybrid polypeptides comprising Ii-key motif and MHC class I or II-presented epitope of antigen, allergen or tumor antigen as vaccines against infection, allergy and cancer. Humphreys, Robert E.; Xu, Minzhen (Antigen Express, Inc., USA). U.S. Pat. Appl. Publ. US 2004058881 A1 20040325, 90 pp. (English). CODEN: USXXCO. APPLICATION: US 2002-253286 20020924.

AB Disclosed is a nucleic acid mol. comprising a first expressible sequence encoding a protein of interest or polypeptide of interest which contains an MHC Class II-presented epitope. In addition, the nucleic acid mol. comprises a second expressible nucleic acid sequence encoding an antigen presentation-enhancing hybrid polypeptide. The antigen presentation enhancing hybrid polypeptide includes the following elements: i) an N-terminal element consisting essentially of 4-16 residues of the mammalian Ii-Key peptide: LRMKLPKPPKPVSKMR and non-N-terminal deletion modifications thereof that retain antigen presentation enhancing activity; ii) a C-terminal element comprising an MHC Class II-presented epitope in the form of a polypeptide or peptidomimetic structure which binds to the antigenic peptide binding site of an MHC class II mol., the MHC Class II-presented epitope being contained in the protein of interest of step a); and iii) an intervening peptidyl structure linking the N-terminal and C-terminal elements of the hybrid, the peptidyl structure having a length of about 20 amino acids or less. Exemplified proteins are allergen: Ara h 1-3, Fel d 1, Phi p 1, Phl p 5a, Bla q 5, and bee venom phospholipase A2; tumor antigen: CEA, CA-125, PSA, gp100, Pmel17, TRP-2, melanoma tyrosinase, MART-1, and Her-2 neu; pathogenic antigen: anthrax toxin lethal factor, anthrax protective antigen, Variola virus B5R protein, and Ebola virus membrane-associated protein VP24; and autoantigen: myelin basic protein, proteolipid protein, and myelin-oligodendrocyte glycoprotein precursor.

L28 ANSWER 4 OF 30 MEDLINE on STN

2004105813. PubMed ID: 14995153. cDNA clone of a putative peanut (Arachis hypogaea L.) trypsin inhibitor has homology with peanut allergens Ara h 3 and Ara h 4. Dodo Hortense W; Viquez Olga M; Maleki Soheila J; Konan Koffi N. (Department of Food and Animal Sciences, Food Biotechnology Laboratory, Alabama A&M University, Normal, Alabama 35762, USA.. aamhwd01@aamu.edu) . Journal of agricultural and food chemistry, (2004 Mar 10) 52 (5) 1404-9.

Journal code: 0374755. ISSN: 0021-8561. Pub. country: United States. Language: English.

AB Trypsin inhibitors are pathogenesis-related (PR) proteins, which play an important role in the plant defense mechanism against insects and pathogens. Peanut trypsin inhibitors are low molecular mass seed storage proteins. Like peanut allergens, they are stable to acid and heat, resistant to digestion, and can have a negative impact on human health. In peanut, five Bowman-Birk trypsin inhibitors (BBTI) have been isolated and amino acid sequences published. However, to date, no peanut BBTI sequence is available at both the cDNA and the genomic levels. The objectives of this investigation were (i) to synthesize degenerate oligonucleotides based on conserved regions of published amino acid sequences of BBTI, BII, and BIII; (ii) to isolate, sequence, and analyze at least one positive peanut trypsin inhibitor cDNA clone using the synthesized (32)P-labeled oligonucleotides as probes; and (iii) to determine its trypsin inhibitory activity. Thirty-two degenerate oligonucleotides DNA primers of 24 nucleotides each were synthesized based on the published amino acid sequences of peanut BBTI, and two were selected as probes to screen a peanut Lambda gt 11 cDNA library. Three putative positive clones were isolated, purified, and subcloned, and one was sequenced. Sequence analysis revealed a partial cDNA clone of 643 bp with a start codon. This clone shares 93 and 96% nucleotide sequence homology with peanut allergens Ara h 3 and Ara h 4 cDNA clones, respectively. A trypsin inhibitor assay revealed that peanut allergen Ara h 3 has a trypsin inhibitory activity of 11 238 TIA/mg protein. We concluded that peanut allergen Ara h 3 may also function as a trypsin inhibitor.

L28 ANSWER 5 OF 30 MEDLINE on STN

2004513393. PubMed ID: 15482859. Genomic organization of peanut allergen gene, Ara h 3. Viquez Olga M; Konan Koffi N; Dodo Hortense W. (Food Biotechnology Laboratory, Department of Food and Animal Sciences, P.O. Box 1628, Alabama A and M University, Normal AL 35762, USA.) Molecular immunology, (2004 Nov) 41 (12) 1235-40. Journal code: 7905289. ISSN: 0161-5890. Pub. country: England: United Kingdom. Language: English.

AB Type 1 hypersensitivity to peanut proteins is a well-recognized health problem. Several peanut seed storage proteins have been identified as allergens. Ara h 3, a glycinin protein, is one of the important peanut allergens. Although amino acid and cDNA sequences are available for Ara h 3, there is not information at the genomic level. The objectives of this study were to isolate, sequence, and characterize the genomic clone of peanut allergen, Ara h 3. A peanut genomic library was screened, using two [32P] end-labeled oligonucleotide probes designed based on cDNA sequences of Ara h 3 and Ara h 4. Four positives lambda FIX II clones were obtained after four rounds of screenings. Digestion with Sac I resulted in two fragments of 1.5 and 10 kb hybridizing to the probes. Both fragments were subcloned into p-Bluescript vector and sequenced. The Ara h 3 gene spans 3.5 kb and consists of four exons, three introns, 5' and 3' flanking regions. The open reading frame is 2008 bp long and can encode a polypeptide of 538 amino acids residues. Sequences analogous to a TATA-box (TATAAAT), CAAT-box (AGGA), G-box (TCCTACGTGTCC) and several cis-elements were found in the promoter region. In the 3' downstream region, three polyadenylation signals (AATAAA) were identified.

L28 ANSWER 6 OF 30 MEDLINE on STN

2004202343. PubMed ID: 15100687. Microarray immunoassay: association of clinical history, in vitro IgE function, and heterogeneity of allergenic peanut epitopes. Shreffler Wayne G; Beyer Kirsten; Chu Te-Hua Tearina; Burks A Wesley; Sampson Hugh A. (Jaffe Food Allergy Institute, Division of Allergy and Immunology, Department of Pediatrics, Mount Sinai Medical Center, Box 1198, One Gustave L. Levy Place, New York, NY 10029, USA.) Journal of allergy and clinical immunology, (2004 Apr) 113 (4) 776-82. Journal code: 1275002. ISSN: 0091-6749. Pub. country: United States. Language: English.

AB BACKGROUND: IgE epitope mapping of food allergens is a prerequisite for

engineering hypoallergenic immunotherapeutic agents and might reveal basic information regarding a patient's immune response. Mapping of large numbers of epitopes by using individual patient sera has been impractical with current techniques. OBJECTIVE: We sought to develop a peptide microarray-based immunoassay to map peanut epitopes by using microliter quantities of serum. METHODS: A set of 213 overlapping 20-residue peptides was synthesized corresponding to the primary sequences of Ara h 1, Ara h 2, and Ara h 3. These were arrayed in triplicate along with the corresponding recombinant proteins onto glass slides and used for immunolabeling. RESULTS: Seventy-seven patient and 15 control sera were analyzed. The majority of patients (97%) had specific IgE to at least one of the recombinant allergens, and 87% had detectable IgE to sequential epitopes. Microarray mapping correlated well with previous studies. However, the analysis of individual patients revealed remarkable heterogeneity in the number and patterns of epitope recognition. High epitope diversity was found in patients with a history of more severe allergic reactions. Also, sensitization of effector cells with more diverse IgE antibodies conferred greater reactivity to specific allergen. CONCLUSIONS: The protein microarray immunoassay confirmed that Ara h 1, Ara h 2, and Ara h 3 are major peanut allergens and allows for parallel epitope analysis. This has led to the discovery of an additional important epitope of Ara h 1 and the recognition of a high degree of patient heterogeneity. This qualitative difference in epitope diversity might provide prognostic information about the patient.

L28 ANSWER 7 OF 30 MEDLINE on STN DUPLICATE 1

2004185497. PubMed ID: 15080811. Relevance of Ara h1, Ara h2 and **Ara h3** in peanut-allergic patients, as determined by immunoglobulin E Western blotting, basophil-histamine release and intracutaneous testing: Ara h2 is the most important peanut allergen. Koppelman S J; Wensing M; Ertmann M; Knulst A C; Knol E F. (TNO Nutrition and Food Research, Zeist, The Netherlands.. koppelman@voeding.tno.nl) . Clinical and experimental allergy : journal of the British Society for Allergy and Clinical Immunology, (2004 Apr) 34 (4) 583-90. Journal code: 8906443. ISSN: 0954-7894. Pub. country: England: United Kingdom. Language: English.

AB BACKGROUND: A number of allergenic proteins in peanut has been described and the relative importance of these allergens is yet to be determined. OBJECTIVES: We have investigated the relevance of previously identified peanut allergens in well-characterized peanut-allergic patients by in vitro, ex vivo and in vivo assays. METHODS: Thirty-two adult peanut-allergic patients were included based on careful and standardized patient history and the presence of peanut-specific IgE. The diagnosis peanut allergy was confirmed using double-blind placebo-controlled food challenges in 23 patients. Major peanut allergens Ara h1, Ara h2 and **Ara h3** were purified from peanuts using ion-exchange chromatography. IgE immunoblotting was performed and IgE-cross-linking capacity was examined by measuring histamine release (HR) after incubating patient basophils as well as passively sensitized basophils with several dilutions of the allergens. Intracutaneous tests (ICTs) using 10-fold dilution steps of the purified allergens and crude peanut extract were performed. RESULTS: Ara h2 was recognized most frequently (26 out of 32) in all tests and induced both positive skin tests and basophil degranulation at low concentrations, whereas Ara h1 and **Ara h3** were recognized less frequently and reacted only at 100-fold higher concentrations as analysed with HR and intracutaneous testing (ICT). Next to the three tested allergens, proteins with molecular weights of somewhat smaller than 15 kDa were identified as a IgE-binding proteins on immunoblot in the majority of the patients (20 out of 32). CONCLUSION: We conclude that Ara h2 is, for our patient group, the most important peanut allergen, and that previously unidentified peanut proteins with molecular weights of somewhat smaller than 15 kDa may be important allergens as well. ICT in combination with basophil-HR and IgE immunoblotting provides insight in the patient specificity towards the individual peanut allergens.

L28 ANSWER 8 OF 30 BIOSIS COPYRIGHT (c) 2005 The Thomson Corporation on STN
2004:209261 Document No.: PREV200400210116. The role of CTLA-4 and a mucosal adjuvant cholera toxin in oral sensitization to peanut. van Wijk, F. [Reprint Author]; Hoeks, S. [Reprint Author]; Knippels, L.; Boon, L.; Koppelman, S.; Pieters, R. [Reprint Author]. Immunotoxicology, Institute for Risk Assessment Sciences, Utrecht, Netherlands. Journal of Allergy and Clinical Immunology, (February 2004) Vol. 113, No. 2 Supplement, pp. S234-S235. print.
Meeting Info.: 60th Annual Meeting of the American Academy of Allergy, Asthma and Immunology (AAAAI). San Francisco, CA, USA. March 19-23, 2004. American Academy of Allergy, Asthma and Immunology.
CODEN: JACIBY. ISSN: 0091-6749. Language: English.

L28 ANSWER 9 OF 30 EMBASE COPYRIGHT 2005 ELSEVIER INC. ALL RIGHTS RESERVED. on STN
2005043691 EMBASE Cloning and structural analysis of a cDNA clone encoding glycinin (Gly-1) seed storage protein of peanut. Jain A.K.. A.K. Jain, Environmental Biotechnology Lab., Dyson Bldg., Coll. Pharm. and Pharmaceutical Sci., Tallahassee, FL 32307, United States. ashok.jain@famu.edu. Electronic Journal of Biotechnology Vol. 7, No. 3, pp. 221-231 15 Dec 2004.
Refs: 44.
ISSN: 0717-3458. Pub. Country: Chile. Language: English. Summary Language: English.
ED Entered STN: 20050204
AB A cDNA clone (peanut Gly-1) encoding for glycinin protein was isolated from the immature seeds (from yellow-1 maturity pods) and characterized. The clone spanning a total of 1836 bp, predicted protein of 529 amino acid residues with a calculated mass of 60,447.61 Da. Peanut Gly-1 sequence comparison shows high level of sequence homology with other two peanut glycinin (arachin) genes [**Ara h3** (95%) and Ara h4 (94%)] and glycinin (legumin) genes of other legumes such as soybean, broad bean, French bean and pea etc., both at nucleotide (67 to 69%) and amino acid (60 to 63%) levels. The N- and C-terminals of peanut Gly-1 are highly conserved with other glycinin genes; central region of the gene possess three variable regions, which also show conservation with other glycinin genes. peanut glycinin-1 gene deciphers 11S type A seed storage protein. Mapping for conserved domains indicate that peanut Gly-1 consists of bi-cupin domain. RNA gel blot studies demonstrate that the gene expressed during embryo development that is transcriptionally activated early in embryogenesis (white pod maturity) and is repressed late in seed maturation (orange pod maturity stage). Peanut Gly-1 does not express in other tissues like leaf, stem, root, flower, pegs or post germinating seedlings.

L28 ANSWER 10 OF 30 CAPLUS COPYRIGHT 2005 ACS on STN
2005:154352 Cloning and structural analysis of a cDNA clone encoding glycinin (Gly-1) seed storage protein of peanut. Jain, Ashok K. (Plant Biotechnol. Lab., Coll. Eng. Sci., Technol. and Agriclture, Florida A& M Univ., Tallahassee, FL, 32307, USA). Electronic Journal of Biotechnology, 7(3), No pp. given (English) 2004. CODEN: EEBIF6. ISSN: 0717-3458. URL: http://www.ejbiotechnology.info/content/vol7/issue3/full/13/13.pdf
Publisher: Universidad Catolica de Valparaiso.
AB A cDNA clone (peanut Gly-1) encoding for glycinin protein was isolated from the immature seeds (from yellow-1 maturity pods) and characterized. The clone spanning a total of 1836 bp, predicted protein of 529 amino acid residues with a calculated mass of 60,447.61 Da. Peanut Gly-1 sequence comparison shows high level of sequence homol. with other two peanut glycinin (arachin) genes [**Ara h3** (95%) and Ara h4 (94%)] and glycinin (legumin) genes of other legumes such as soybean, broad bean, French bean and pea etc., both at nucleotide (67 to 69%) and amino. acid (60 to 63%) levels. The N- and C-terminals of peanut Gly-1 are highly conserved with other glycinin genes; central region of the gene possess three variable regions, which also show conservation with other

glycinin genes. peanut glycinin-1 gene deciphers 11S type A seed storage protein. Mapping for conserved domains indicate that peanut Gly-1 consists of bi-cupin domain. RNA gel blot studies demonstrate that the gene expressed during embryo development that is transcriptionally activated early in embryogenesis (white pod maturity) and is repressed late in seed maturation (orange pod maturity stage). Peanut Gly-1 does not express in other tissues like leaf, stem, root, flower, pegs or post germinating seedlings.

L28 ANSWER 11 OF 30 MEDLINE on STN

2003119082. PubMed ID: 12626588. Engineered recombinant peanut protein and heat-killed Listeria monocytogenes coadministration protects against peanut-induced anaphylaxis in a murine model. Li Xiu-Min; Srivastava Kamal; Huleatt James W; Bottomly Kim; Burks A Wesley; Sampson Hugh A. (Department of Pediatrics, Mount Sinai School of Medicine, New York, NY 10029, USA.. xiu-min.li@mssm.edu) . Journal of immunology (Baltimore, Md. : 1950), (2003 Mar 15) 170 (6) 3289-95. Journal code: 2985117R. ISSN: 0022-1767. Pub. country: United States. Language: English.

AB Peanut allergy (PNA) is the major cause of fatal and near-fatal anaphylactic reactions to foods. Traditional immunotherapy using peanut (PN) protein is not an option for PNA therapy because of the high incidence of adverse reactions. We investigated the effects of s.c. injections of engineered (modified) recombinant PN proteins and heat-killed Listeria monocytogenes (HKLM) as an adjuvant on anaphylactic reactions in a mouse model of PN allergy. PN-allergic C3H/HeJ mice were treated s.c. with a mixture of the three major PN allergens and HKLM (modified (m)Ara h 1-3 plus HKLM). The effects on anaphylactic reactions following PN challenge and the association with Ab levels and cytokine profiles were determined. Although all mice in the sham-treated groups exhibited anaphylactic symptoms with a median symptom score of 3, only 31% of mice in the mAra h 1-3 plus HKLM group developed mild anaphylaxis, with a low median symptom score of 0.5. Alterations in core body temperature, bronchial constriction, plasma histamine, and PN-specific IgE levels were all significantly reduced. This protective effect was markedly more potent than in the mAra h 1-3 protein alone-treated group. HKLM alone did not have any protective effect. Reduced IL-5 and IL-13, and increased IFN-gamma levels were observed only in splenocytes cultures from mAra h 1-3 plus HKLM-treated mice. These results show that immunotherapy with modified PN proteins and HKLM is effective for treating PN allergy in this model, and may be a potential approach for treating PNA.

L28 ANSWER 12 OF 30 MEDLINE on STN

2003546870. PubMed ID: 14616125. Peanut allergen Ara h 3: isolation from peanuts and biochemical characterization. Koppelman S J; Knol E F; Vlooswijk R A A; Wensing M; Knulst A C; Hefle S L; Gruppen H; Piersma S. (TNO Nutrition and Food Research Institute, Zeist, The Netherlands.) Allergy, (2003 Nov) 58 (11) 1144-51. Journal code: 7804028. ISSN: 0105-4538. Pub. country: Denmark. Language: English.

AB BACKGROUND: Peanut allergen Ara h 3 has been the subject of investigation for the last few years. The reported data strongly depend on recombinant Ara h 3, since a purification protocol for Ara h 3 from peanuts was not available. METHODS: Peanut allergen Ara h 3 (glycinin), was purified and its posttranslational processing was investigated. Its allergenic properties were determined by studying IgE binding characteristics of the purified protein. RESULTS: Ara h 3 consists of a series of polypeptides ranging from approximately 14 to 45 kDa that can be classified as acidic and basic subunits, similar to the subunit organization of soy glycinin. N-terminal sequences of the individual polypeptides were determined, and using the cDNA deduced amino-acid sequence, the organization into subunits was explained by revealing posttranslational processing of the different polypeptides. IgE-binding properties of Ara h 3 were investigated using direct elisa and Western blotting with sera from peanut-allergic individuals. The basic subunits, and to a lesser extent the acidic subunits, bind IgE and may act as allergenic peptides. CONCLUSIONS: We conclude that peanut-derived Ara h 3, in contrast to earlier reported

recombinant Ara h 3, resembles, to a large extent, the molecular organization typical for proteins from the glycinin family. Furthermore, posttranslational processing of Ara h 3 affects the IgE-binding properties and is therefore an essential subject of study for research on the allergenicity of Ara h 3.

L28 ANSWER 13 OF 30 BIOSIS COPYRIGHT (c) 2005 The Thomson Corporation on STN

2003:239426 Document No.: PREV200300239426. Silencing the three major allergens for the production of hypoallergenic peanut. Konan, K. N. [Reprint Author]; Viquez, O. M. [Reprint Author]; Dodo, H. W. [Reprint Author]. Alabama A and M University, Normal, AL, USA. Journal of Allergy and Clinical Immunology, (April 2003) Vol. 111, No. 4, pp. 909. print. Meeting Info.: 60th Anniversary Meeting of the American Academy of Allergy, Asthma and Immunology (AAAAI). Denver, CO, USA. March 07-12, 2003. American Academy of Allergy, Asthma and Immunology. CODEN: JACIBY. ISSN: 0091-6749. Language: English.

L28 ANSWER 14 OF 30 MEDLINE on STN

2004221558. PubMed ID: 15119044. New perspectives for the treatment of food allergy (peanut). Sampson Hugh A; Srivastava Kamal; Li Xiu-Min; Burks A Wesley. (Jaffe Food Allergy Institute, Department of Pediatrics, Division of Allergy and Immunology, Mount Sinai School of Medicine, Box 1198, One Gustave L. Levy Pl., New York, Ny. 10029, USA.) Arbeiten aus dem Paul-Ehrlich-Institut (Bundesamt fur Sera und Impfstoffe) zu Frankfurt a.M, (2003) (94) 236-44; discussion 244-6. Journal code: 8912864. ISSN: 0936-8671. Pub. country: Germany: Germany, Federal Republic of. Language: English.

L28 ANSWER 15 OF 30 MEDLINE on STN

2003342840. PubMed ID: 12847500. Measurement of peptide-specific IgE as an additional tool in identifying patients with clinical reactivity to peanuts. Beyer Kirsten; Ellman-Grunther Lisa; Jarvinen Kirsi-Marjut; Wood Robert A; Hourihane Jonathan; Sampson Hugh A. (Division of Pediatric Allergy & Immunology and Jaffe Institute for Food Allergy, The Mount Sinai School of Medicine, New York, NY 10029-6574, USA.) Journal of allergy and clinical immunology, (2003 Jul) 112 (1) 202-7. Journal code: 1275002. ISSN: 0091-6749. Pub. country: United States. Language: English.

AB BACKGROUND: Peanut allergy is one of the most common food allergies, often resulting in severe reactions. Diagnostic decision levels of food-specific IgE antibody concentrations have been described. However, many patients still need to undergo oral peanut challenges because their IgE levels are in the nondiagnostic level. OBJECTIVE: The aim of this study was to determine whether differences exist in IgE-binding epitope recognition between sensitized children with and without symptomatic peanut allergy. METHODS: Eight peptides representing the immunodominant sequential epitopes on Ara h 1, 2, and 3 were synthesized on SPOTs membranes. Individual patient labeling was performed with sera from 15 patients with symptomatic peanut allergy and 16 patients who were sensitized but tolerant. Ten of these 16 patients had "outgrown" their allergy. RESULTS: Regardless of their peanut-specific IgE levels, most patients with symptomatic peanut allergy showed IgE binding to the 3 immunodominant epitopes on Ara h 2. In contrast, each of these epitopes was recognized by < 10% of the tolerant patients. In addition, tolerant patients did not recognize 2 immunodominant epitopes on Ara h 1. At least 93% of symptomatic, but only 12.5% of tolerant patients, recognized 1 of these "predictive" epitopes on Ara h 1 or 2. Moreover, the cumulative IgE binding to the peanut peptides was significantly higher in patients with peanut allergy than in tolerant patients. With up to 50% of patients with peanut-specific IgE levels below diagnostic decision levels still being clinically reactive, oral food challenges could be avoided in 90% of these patients through determination of peptide-specific IgE. CONCLUSIONS: Determination of epitope recognition provides an additional tool to diagnose symptomatic peanut allergy, especially in children with peanut-specific IgE below diagnostic decision levels.

L28 ANSWER 16 OF 30 BIOSIS COPYRIGHT (c) 2005 The Thomson Corporation on STN

2003:347651 Document No.: PREV200300347651. Relevance of Ara h1, Ara h2, and **Ara h3** in peanut allergic patients, as determined by IgE-western-blotting, basophil histamine release, and intracutaneous testing: Ara h2 is the most important peanut allergen. Knol, E. F. [Reprint Author]; Wensing, M. [Reprint Author]; Vlooswijk, R.; Ertmann, M. [Reprint Author]; Knulst, A. C. [Reprint Author]; Koppelman, S. J.. Dermatology/Allergology, University Medical Center Utrecht, Utrecht, Netherlands. Journal of Allergy and Clinical Immunology, (February 2003) Vol. 111, No. 2 Abstract Supplement, pp. S194-S195. print.
Meeting Info.: AAAAI 60th Anniversary Meeting. Denver, CO, USA. March 07-12, 2003. American Academy of Allergy, Asthma and Immunology.
CODEN: JACIBY. ISSN: 0091-6749. Language: English.

L28 ANSWER 17 OF 30 MEDLINE on STN

2003342838. PubMed ID: 12847493. Persistent protective effect of heat-killed Escherichia coli producing "engineered," recombinant peanut proteins in a murine model of peanut allergy. Li Xiu-Min; Srivastava Kamal; Grishin Alexander; Huang Chih-Kang; Schofield Brian; Burks Wesley; Sampson Hugh A. (Department of Pediatrics, Mount Sinai School of Medicine, New York, NY 10029-6574, USA.) Journal of allergy and clinical immunology, (2003 Jul) 112 (1) 159-67. Journal code: 1275002. ISSN: 0091-6749. Pub. country: United States. Language: English.

AB BACKGROUND: Peanut allergy (PNA) is a life-threatening food allergy for which there is no definitive treatment. OBJECTIVE: We investigated the long-term immunomodulatory effect of heat-killed Escherichia coli producing engineered (mutated) Ara h1, 2, and 3 (HKE-MP123) administered rectally (pr) in a murine model of PNA. METHODS: Peanut-allergic C3H/HeJ mice received 0.9 (low dose), 9 (medium dose), or 90 (high dose) microg HKE-MP123 pr, HKE-containing vector (HKE-V) alone, or vehicle alone (sham) weekly for 3 weeks. Mice were challenged 2 weeks later. A second and third challenge were performed at 4-week intervals. RESULTS: After the first challenge, all 3 HKE-MP123 and HKE-V-treated groups exhibited reduced symptom scores (P <.01,.01,.05,.05, respectively) compared with the sham-treated group. Interestingly, only the medium- and high-dose HKE-MP123-treated mice remained protected for up to 10 weeks after treatment accompanied by a significant reduction of plasma histamine levels compared with sham-treated mice (P <.05 and.01, respectively). IgE levels were significantly lower in all HKE-MP123-treated groups (P <.001), being most reduced in the high-dose HKE-MP123-treated group at the time of each challenge. IL-4, IL-13, IL-5, and IL-10 production by splenocytes of high-dose HKE-MP123-treated mice were significantly decreased (P <.01;.001,.001, and.001, respectively), and IFN-gamma and TGF-beta production were significantly increased (P <.001 and.01, respectively) compared with sham-treated mice at the time of the last challenge. CONCLUSIONS: Treatment with pr HKE-MP123 can induce long-term "downregulation" of peanut hypersensitivity, which might be secondary to decreased antigen-specific T(H)2 and increased T(H)1 and T regulatory cytokine production.

L28 ANSWER 18 OF 30 BIOSIS COPYRIGHT (c) 2005 The Thomson Corporation on STN

2003:209478 Document No.: PREV200300209478. Polyisotypic antibody and mixed cytokine responses to the major peanut allergens in an oral mouse food allergy model. van Wijk, F. [Reprint Author]; Hartgring, S. [Reprint Author]; Knippels, L. M.; Pieters, R. [Reprint Author]. Immunotoxicology, IRAS, Utrecht, Netherlands. Toxicological Sciences, (March 2003) Vol. 72, No. S-1, pp. 51. print.
Meeting Info.: 42nd Annual Meeting of the Society of Toxicology. Salt Lake City, Utah, USA. March 09-13, 2003. Society of Toxicology.
ISSN: 1096-6080 (ISSN print). Language: English.

L28 ANSWER 19 OF 30 MEDLINE on STN

2002453715. PubMed ID: 12209105. Identification of an 11S globulin as a major hazelnut food allergen in hazelnut-induced systemic reactions. Beyer Kirsten; Grishina Galina; Bardina Ludmilla; Grishin Alexander; Sampson Hugh A. (Division of Pediatric Allergy and Immunology and the Jaffe Institute for Food Allergy, The Mount Sinai School of Medicine, New York, NY 10029, USA.) Journal of allergy and clinical immunology, (2002 Sep) 110 (3) 517-23. Journal code: 1275002. ISSN: 0091-6749. Pub. country: United States. Language: English.

AB BACKGROUND: Hazelnuts are a common cause of food allergy. Allergic reactions to hazelnuts range from oral allergy syndrome caused by cross-reactivity between tree pollen and hazelnut proteins to severe anaphylactic reactions. Little information is available regarding the identification of pollen-independent hazelnut allergens. OBJECTIVE: The aim of the study was to identify these pollen-independent allergens in patients with hazelnut allergy with systemic reactions. METHODS: Extracted hazelnut proteins were separated by means of 2-dimensional PAGE, and immunolabeling was performed with individual sera from 14 patients with hazelnut-induced systemic reactions. Edman sequencing was performed on a 40-kd protein identified as an allergen. In parallel, RNA isolated from hazelnuts was used to construct a cDNA library. By using the peptide sequence data, oligonucleotide primers were synthesized and used to screen the library. Full-length cDNA clones were isolated, sequenced, expressed, and screened with patient sera. RESULTS: By using 2-dimensional proteomics, a protein fraction at 40 kd was recognized by serum IgE from 86% (12/14) of the patients with hazelnut allergy with systemic reactions. Two internal amino acid sequences were determined by means of Edman sequencing. Screening of the prepared hazelnut cDNA library with oligonucleotides based on these internal peptide sequences resulted in isolation of a novel protein cDNA. The new protein, named Cor a 9, belongs to the 11S globulin seed storage protein family. This family comprises known food allergens in peanut (Ara h 3) and soybean (glycine max). The pairwise homology among these 3 proteins ranges from 45% to 50%. Interestingly, one known IgE-binding epitope of Ara h 3 shares 67% of homologous amino acid residues with the corresponding area of Cor a 9. The amino acids that differ were previously shown not to be critical for IgE binding in Ara h 3. CONCLUSION: Cor a 9 is the first tree pollen-unrelated hazelnut allergen isolated, sequenced, and cloned. The identification of food allergens is the first step toward generating recombinant allergens for use in future immunotherapeutic approaches. In addition, the detection of conserved IgE epitopes in common food allergens, such as seed storage proteins, might be a useful tool for predicting cross-reactivity to certain foods.

L28 ANSWER 20 OF 30 BIOSIS COPYRIGHT (c) 2005 The Thomson Corporation on STN

2002:301398 Document No.: PREV200200301398. Immunotherapy for peanut allergy using modified allergens and a bacterial adjuvant. Stanley, Joseph Steve [Reprint author]; Buzen, Fred [Reprint author]; Cockrell, Gael [Reprint author]; West, Mike [Reprint author]; Srivastava, Kamal D.; Li, X. M.; Sampson, Hugh A.; Burks, Wesley [Reprint author]; Bannon, Gary A. [Reprint author]. University of Arkansas, Little Rock, AR, USA. Journal of Allergy and Clinical Immunology, (January, 2002) Vol. 109, No. 1 Supplement, pp. S93. print.
Meeting Info.: 58th Annual Meeting of the American Academy of Allergy, Asthma and Immunology. New York, NY, USA. March 01-06, 2002. American Academy of Allergy, Asthma, and Immunology.
CODEN: JACIBY. ISSN: 0091-6749. Language: English.

L28 ANSWER 21 OF 30 MEDLINE on STN

2002722819. PubMed ID: 12485602. Identification and analysis of a conserved immunoglobulin E-binding epitope in soybean G1a and G2a and peanut Ara h 3 glycinins. Xiang Ping; Beardslee Tom A; Zeece Michael G; Markwell John; Sarath Gautam. (Department of Biochemistry, University of Nebraska-Lincoln, Lincoln, NE 68588-0664, USA.) Archives of biochemistry and biophysics, (2002 Dec 1) 408 (1) 51-7. Journal code: 0372430. ISSN:

0003-9861. Pub. country: United States. Language: English.

AB To identify conserved immunoglobulin E (IgE)-binding epitopes among legume glycinins, we utilized recombinant soybean G2a and G2a-derived polypeptide fragments. All of these fusion polypeptides bound IgE, and the C-terminal 94-residue fragment appeared to bind more IgE. Using synthetic peptides we identified S219-N233 (S(219)GFAPEFLKEAFGVN(233)) as the dominant IgE-binding epitope. Alanine scanning of this epitope indicated that six amino acids (E224, F225, L226, F230, G231, and V232) contributed most to IgE binding. Among these amino acids, only G231 of soybean G2a is not conserved in soybean G1a (S234) and peanut Ara h 3 (Q256). Synthetic peptides corresponding to the equivalent regions in G1a and Ara h 3 bound IgE in the order Ara h 3>/=soybean G2a>soybean G1a. This sequence represents a new IgE-binding epitope that occurs in a highly conserved region present in legume glycinins. Such IgE-binding sites could provide a molecular explanation for the IgE cross-reactivity observed between soybean and peanut proteins.

L28 ANSWER 22 OF 30 MEDLINE on STN

2002296618. PubMed ID: 12037397. Modification of peanut allergen Ara h 3: effects on IgE binding and T cell stimulation. Rabjohn Pat; West C Michael; Connaughton Cathie; Sampson Hugh A; Helm Ricki M; Burks A Wesley; Bannon Gary A. (Department of Biochemistry and Molecular Biology, Arkansas Children's Hospital Research Institute, University of Arkansas for Medical Sciences, Little Rock, AR 72202, USA.) International archives of allergy and immunology, (2002 May) 128 (1) 15-23. Journal code: 9211652. ISSN: 1018-2438. Pub. country: Switzerland. Language: English.

AB BACKGROUND: Peanut allergy is a major health concern due to the increased prevalence, potential severity, and chronicity of the reaction. The cDNA encoding a third peanut allergen, Ara h 3, has been previously cloned and characterized. Mutational analysis of the Ara h 3 IgE-binding epitopes with synthetic peptides revealed that single amino acid changes at critical residues could diminish IgE binding. METHODS: Specific oligonucleotides were used in polymerase chain reactions to modify the cDNA encoding Ara h 3 at critical IgE binding sites. Four point mutations were introduced into the Ara h 3 cDNA at codons encoding critical amino acids in epitopes 1, 2, 3 and 4. Recombinant modified proteins were used in SDS-PAGE/Western IgE immunoblot, SDS-PAGE/Western IgE immunoblot inhibition and T cell proliferation assays to determine the effects of these changes on in vitro clinical indicators of peanut hypersensitivity. RESULTS: Higher amounts of modified Ara h 3 were required to compete with the wild-type allergen for peanut-specific serum IgE. Immunoblot analysis with individual serum IgE from Ara-h-3-allergic patients showed that IgE binding to the modified protein decreased approximately 35-85% in comparison to IgE binding to wild-type Ara h 3. Also, the modified Ara h 3 retained the ability to stimulate T cell activation in PBMCs donated by Ara-h-3-allergic patients. CONCLUSIONS: The engineered hypoallergenic Ara h 3 variant displays two characteristics essential for recombinant allergen immunotherapy; it has a reduced binding capacity for serum IgE from peanut-hypersensitive patients and it can stimulate T-cell proliferation and activation.

L28 ANSWER 23 OF 30 CAPLUS COPYRIGHT 2005 ACS on STN

2001:380762 Document No. 135:1229 Isolation and sequence of a full length genomic clone for allergen Ara h2 and down-regulation and silencing of allergen genes in transgenic peanut seeds. Dodo, Hortense W.; Arntzen, Charles J.; Konan, Koffi N'da; Viquez, Olga M. (Alabama A + M University, USA). PCT Int. Appl. WO 2001036621 A2 20010525, 73 pp. DESIGNATED STATES: W: AE, AG, AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, BZ, CA, CH, CN, CR, CU, CZ, DE, DK, DM, DZ, EE, ES, FI, GB, GD, GE, GH, GM, HR, HU, ID, IL, IN, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MA, MD, MG, MK, MN, MW, MX, MZ, NO, NZ, PL, PT, RO, RU, SD, SE, SG, SI, SK, SL, TJ, TM, TR, TT, TZ, UA, UG, US, UZ, VN, YU, ZA, ZW, AM, AZ, BY, KG, KZ, MD, RU, TJ, TM; RW: AT, BE, BF, BJ, CF, CG, CH, CI, CM, CY, DE, DK, ES, FI, FR, GA, GB, GR, IE, IT, LU, MC, ML, MR, NE, NL, PT, SE, SN, TD,

TG, TR. (English). CODEN: PIXXD2. APPLICATION: WO 2000-US31657 20001120. PRIORITY: US 1999-PV167255 19991119.

AB An allergen-free transgenic peanut seed is produced by recombinant methods. Peanut plants are transformed with multiple copies of each of the allergen genes, or fragments thereof, to suppress gene expression and allergen protein production Alternatively, peanut plants are transformed with peanut allergen antisense genes introduced into the peanut genome as antisense fragments, sense fragments, or combinations of both antisense and sense fragments. Peanut transgenes are under the control of the 35S promoter, or the promoter of the Ara h2 gene to produce antisense RNAs, sense RNAs, and double-stranded RNAs for suppressing allergen protein production in peanut plants. A full length genomic clone for allergen Ara h2 is isolated and sequenced. The ORF is 622 nucleotides long. The predicted encoded protein is 207 amino acids long and includes a putative transit peptide of 21 residues. One polyadenilation signal is identified at position 951. Six addnl. stop codons are observed A promoter region was revealed containing a putative TATA box located at position -72. Homologous regions were identified between Ara h2, h6, and h7, and between **Ara h3** and h4, and between Ara h1P41B and Ara h1P17. The homologous regions will be used for the screening of peanut genomic library to isolate all peanut allergen genes and for down-regulation and silencing of multiple peanut allergen genes.

L28 ANSWER 24 OF 30 MEDLINE on STN DUPLICATE 2

2001248467. PubMed ID: 11274350. A strategy for the identification of proteins targeted by thioredoxin. Yano H; Wong J H; Lee Y M; Cho M J; Buchanan B B. (Department of Plant and Microbial Biology, University of California, Berkeley, CA 94720, USA.) Proceedings of the National Academy of Sciences of the United States of America, (2001 Apr 10) 98 (8) 4794-9. Electronic Publication: 2001-03-27. Journal code: 7505876. ISSN: 0027-8424. Pub. country: United States. Language: English.

AB Thioredoxins are 12-kDa proteins functional in the regulation of cellular processes throughout the animal, plant, and microbial kingdoms. Growing evidence with seeds suggests that an h-type of thioredoxin, reduced by NADPH via NADP-thioredoxin reductase, reduces disulfide bonds of target proteins and thereby acts as a wakeup call in germination. A better understanding of the role of thioredoxin in seeds as well as other systems could be achieved if more were known about the target proteins. To this end, we have devised a strategy for the comprehensive identification of proteins targeted by thioredoxin. Tissue extracts incubated with reduced thioredoxin are treated with a fluorescent probe (monobromobimane) to label sulfhydryl groups. The newly labeled proteins are isolated by conventional two-dimensional electrophoresis: (i) nonreducing/reducing or (ii) isoelectric focusing/reducing SDS/PAGE. The isolated proteins are identified by amino acid sequencing. Each electrophoresis system offers an advantage: the first method reveals the specificity of thioredoxin in the reduction of intramolecular vs. intermolecular disulfide bonds, whereas the second method improves the separation of the labeled proteins. By application of both methods to peanut seed extracts, we isolated at least 20 thioredoxin targets and identified 5-three allergens (Ara h2, **Ara h3**, and Ara h6) and two proteins not known to occur in peanut (desiccation-related and seed maturation protein). These findings open the door to the identification of proteins targeted by thioredoxin in a wide range of systems, thereby enhancing our understanding of its function and extending its technological and medical applications.

L28 ANSWER 25 OF 30 CAPLUS COPYRIGHT 2005 ACS on STN

2001:493625 Document No. 135:287805 Effects of cooking methods on peanut allergenicity. Beyer, Kirsten; Morrow, Ellen; Li, Xiu-Min; Bardina, Ludmilla; Bannon, Gary A.; Burks, A. Wesley; Sampson, Hugh A. (Division of Pediatric Allergy & Immunology and Jaffe Institute for Food Allergy, The Mount Sinai School of Medicine, New York, NY, 10029-6574, USA). Journal of Allergy and Clinical Immunology, 107(6), 1077-1081 (English) 2001. CODEN: JACIBY. ISSN: 0091-6749. Publisher: Mosby, Inc..

AB Allergy to peanut is a significant health problem. Interestingly, the prevalence of peanut allergy in China is much lower than that in the United States, despite a high rate of peanut consumption in China. In China, peanuts are commonly fried or boiled, whereas in the United States peanuts are typically dry roasted. The aim of this study was to examine whether the method of preparing peanuts could be a factor in the disparity of allergy prevalence between the 2 countries. Two varieties of peanuts grown in the United States were roasted, boiled, or fried. Proteins were analyzed by using SDS-PAGE and immunoblotting. Allergenicity was compared by using immunolabeling with sera from 8 patients with peanut allergy. The protein fractions of both varieties of peanuts were altered to a similar degree by frying or boiling. Compared with roasted peanuts, the relative amount of Ara h 1 was reduced in the fried and boiled prepns., resulting in a significant reduction of IgE-binding intensity. In addition, there was significantly less IgE binding to Ara h 2 and Ara h 3 in fried and boiled peanuts compared with that in roasted peanuts, even though the protein amts. were similar in all 3 prepns. The methods of frying or boiling peanuts, as practiced in China, appear to reduce the allergenicity of peanuts compared with the method of dry roasting practiced widely in the United States. Roasting uses higher temps. that apparently increase the allergenic property of peanut proteins and may help explain the difference in prevalence of peanut allergy observed in the 2 countries.

L28 ANSWER 26 OF 30 MEDLINE on STN
2001262411. PubMed ID: 11306930. Engineering, characterization and in vitro efficacy of the major peanut allergens for use in immunotherapy. Bannon G A; Cockrell G; Connaughton C; West C M; Helm R; Stanley J S; King N; Rabjohn P; Sampson H A; Burks A W. (Department of Biochemistry and Molecular Biology, Arkansas Children's Hospital Research Institute, Little Rock 72205, USA.. bannongarya@exchnage.uams.edu) . International archives of allergy and immunology, (2001 Jan-Mar) 124 (1-3) 70-2. Journal code: 9211652. ISSN: 1018-2438. Pub. country: Switzerland. Language: English.
AB BACKGROUND: Numerous strategies have been proposed for the treatment of peanut allergies, but despite the steady advancement in our understanding of atopic immune responses and the increasing number of deaths each year from peanut anaphylaxis, there is still no safe, effective, specific therapy for the peanut-sensitive individual. Immunotherapy would be safer and more effective if the allergens could be altered to reduce their ability to initiate an allergic reaction without altering their ability to desensitize the allergic patient. METHODS: The cDNA clones for three major peanut allergens, Ara h 1, Ara h 2, and Ara h 3, have been cloned and characterized. The IgE-binding epitopes of each of these allergens have been determined and amino acids critical to each epitope identified. Site-directed mutagenesis of the allergen cDNA clones, followed by recombinant production of the modified allergen, provided the reagents necessary to test our hypothesis that hypoallergenic proteins are effective immunotherapeutic reagents for treating peanut-sensitive patients. Modified peanut allergens were subjected to immunoblot analysis using peanut-positive patient sera IgE, T cell proliferation assays, and tested in a murine model of peanut anaphylaxis. RESULTS: In general, the modified allergens were poor competitors for binding of peanut-specific IgE when compared to their wild-type counterpart. The modified allergens demonstrated a greatly reduced IgE-binding capacity when individual patient serum IgE was compared to the binding capacity of the wild-type allergens. In addition, while there was considerable variability between patients, the modified allergens retained the ability to stimulate T cell proliferation. CONCLUSIONS: These modified allergen genes and proteins should provide a safe immunotherapeutic agent for the treatment of peanut allergy.

L28 ANSWER 27 OF 30 MEDLINE on STN DUPLICATE 3
2001119281. PubMed ID: 11146387. Soybean glycinin G1 acidic chain shares IgE epitopes with peanut allergen Ara h 3. Beardslee T A; Zeece M G; Sarath G; Markwell J P. (Department of Biochemistry, University of

Nebraska-Lincoln, Lincoln, NE, USA.. beardsl@yahoo.com) . International archives of allergy and immunology, (2000 Dec) 123 (4) 299-307. Journal code: 9211652. ISSN: 1018-2438. Pub. country: Switzerland. Language: English.

AB BACKGROUND: The identification of IgE epitopes for proteins is the first step in understanding the interaction of allergens with the immune system. Proteins from the legume family have shown in vitro cross-reactivity in IgE-binding assays, but this cross-reactivity is rarely clinically significant. Resolution of this discrepancy requires IgE epitope mapping of legume family protein allergens. METHODS: We constructed six fusion proteins representing overlapping regions of soybean glycinin G1 acidic chain. These fusion proteins were used in immunoblotting and a novel sandwich ELISA with pooled sera from soy-allergic individuals to reveal a common IgE-binding region. This region was the focus for IgE epitope mapping using overlapping synthetic peptides. RESULTS: Data from the fusion protein experiments revealed an IgE-binding region consisting of residues F192-I265. Analysis of the overlapping synthetic peptides to this region indicated that IgE epitopes to glycinin G1 acidic chain consist of residues G217-V235 and G253-I265. The epitopes identified for glycinin G1 acidic chain are homologous to IgE epitopes previously identified for the peanut allergen Ara h 3 [1]. However, residues identified by alanine scanning in the peanut epitopes as being important for IgE binding are different in the natural soybean epitopes. CONCLUSIONS: The IgE epitopes identified for glycinin G1 acidic chain apparently represent an allergenic region of several legume family seed storage proteins. Our findings indicate that the identification of IgE epitopes and structural analysis of legume family proteins will provide valuable information to the study of food allergies.

L28 ANSWER 28 OF 30 CAPLUS

2002:277584 Document No. 136:368007 Peanut allergy: From allergens to immunotherapy. Helm, Ricki M.; Sampson, Hugh A.; Bannon, Gary A.; Burks, A. Wesley (Arkansas Children's Hospital, University of Arkansas for Medical Sciences, Little Rock, AR, 72202, USA). Recent Research Developments in Allergy & Clinical Immunology, 1, 11-21 (English) 2000. CODEN: RRDACJ. Publisher: Research Signpost.

AB A review. Peanut hypersensitivity represents a significant health problem to the peanut sensitive population because of the potential severity, life-long nature, and ubiquitous use of peanut products. The relevant allergens have been identified using serum IgE from peanut-sensitive individuals using physicochem. and immunol. procedures. Ara h 1 and 2 represent major allergens that bind IgE from greater than 90% of the sensitive population. Ara h 3 and other peanut allergens bind serum IgE from less than 50% of the sensitive population. Major linear IgE-binding epitopes have been identified for each of the allergens using recombinant proteins from a cDNA expression library. Mutational anal. of epitopes by alanine substitution indicates that a single amino acid change results in loss of IgE binding. Ara h 1 and 2 are being used to investigate the potential application of vaccine development in the modulation of food allergy. Results from these investigations will allow for the design of new diagnostic and immunotherapeutic approaches to peanut hypersensitivity.

L28 ANSWER 29 OF 30 MEDLINE on STN

1999146968. PubMed ID: 10021462. Molecular cloning and epitope analysis of the peanut allergen Ara h 3. Rabjohn P; Helm E M; Stanley J S; West C M; Sampson H A; Burks A W; Bannon G A. (Department of Biochemistry and Molecular Biology, University of Arkansas for Medical Sciences, Arkansas Children's Hospital Research Institute, Little Rock 72205, USA.) Journal of clinical investigation, (1999 Feb) 103 (4) 535-42. Journal code: 7802877. ISSN: 0021-9738. Pub. country: United States. Language: English.

AB Peanut allergy is a significant IgE-mediated health problem because of the increased prevalence, potential severity, and chronicity of the reaction. Following our characterization of the two peanut allergens Ara h 1 and Ara

h 2, we have isolated a cDNA clone encoding a third peanut allergen, Ara h 3. The deduced amino acid sequence of Ara h 3 shows homology to 11S seed-storage proteins. The recombinant form of this protein was expressed in a bacterial system and was recognized by serum IgE from approximately 45% of our peanut-allergic patient population. Serum IgE from these patients and overlapping, synthetic peptides were used to map the linear, IgE-binding epitopes of Ara h 3. Four epitopes, between 10 and 15 amino acids in length, were found within the primary sequence, with no obvious sequence motif shared by the peptides. One epitope is recognized by all Ara h 3-allergic patients. Mutational analysis of the epitopes revealed that single amino acid changes within these peptides could lead to a reduction or loss of IgE binding. By determining which amino acids are critical for IgE binding, it might be possible to alter the Ara h 3 cDNA to encode a protein with a reduced IgE-binding capacity. These results will enable the design of improved diagnostic and therapeutic approaches for food-hypersensitivity reactions.

L28 ANSWER 30 OF 30 MEDLINE on STN
1999406463. PubMed ID: 10474031. Selective cloning of peanut allergens, including profilin and 2S albumins, by phage display technology. Kleber-Janke T; Crameri R; Appenzeller U; Schlaak M; Becker W M. (Research Center Borstel, Germany.. tkleber@fz-borstel.de) . International archives of allergy and immunology, (1999 Aug) 119 (4) 265-74. Journal code: 9211652. ISSN: 1018-2438. Pub. country: Switzerland. Language: English.
AB BACKGROUND: Peanut kernels contain many allergens able to elicit IgE-mediated type 1 allergic reactions in sensitized individuals. Sera from sensitized patients recognize variable patterns of IgE-binding proteins. The identification of the IgE-binding proteins of peanut extract would faciliate improvement of diagnostic and immunotherapeutic approaches as well as development of sensitive test systems for the detection of hidden peanut allergens present as additives in various industrial food products and the investigation of their stability during processing of food products. METHODS: We applied the pJuFo cloning system based on the phage surface display of functional cDNA expression products to clone cDNAs encoding peanut allergens. Sera (n = 40) of peanut-allergic individuals were selected according to case history, radioallergosorbent test and immunoblot analysis to demonstrate IgE binding towards the newly identified recombinant allergens. RESULTS: In addition to the known allergens Ara h 1 and Ara h 2 we were able to identify four allergens with estimated molecular weights of 36, 16, 14.5 and 14 kDa. Three of them formally termed Ara h 4, Ara h6 and Ara h 7 show significant sequence similarities to the family of seed storage proteins and the fourth (Ara h 5) corresponds to the well-known plant allergen profilin. Immunoblotting of the six expressed recombinant allergens with 40 patients sera shows 14 individual recognition patterns and the following frequency of specific IgE binding: Ara h 1 was recognized by 65%, Ara h 2 by 85%, Ara h 4 by 53%, Ara h 5 by 13%, Ara h 6 by 38% and Ara h 7 by 43% of the selected sera. CONCLUSIONS: All of the selected peanut-positive sera can detect at least one of the six identified recombinant allergens which can be used to establish individual patients' reactivity profiles. A comparison of these profiles with the clinical data will possibly allow a further insight into the relationship between clinical severity of the symptoms and specific IgE levels towards the six peanut allergens.

```
=> s caplan m?/au
L29       1338 CAPLAN M?/AU

=> s l29 and peanut allergen
L30          4 L29 AND PEANUT ALLERGEN

=> dup remove l30
PROCESSING COMPLETED FOR L30
L31          4 DUP REMOVE L30 (0 DUPLICATES REMOVED)
```

=> d l31 1-4 cbib abs

L31 ANSWER 1 OF 4 CAPLUS COPYRIGHT 2005 ACS on STN
2003:855391 Document No. 139:363577 Modified anaphylactic food allergens with reduced IgE-binding ability for decreasing clinical reaction to allergy. **Caplan, Michael J.**; Sosin, Howard B.; Sampson, Hugh; Bannon, Gary A.; Burks, A. Wesley; Cockrell, Gael; Compadre, Cesar M.; Connaughton, Cathie; Helm, Ricki M.; King, Nina E.; Kopper, Randall A.; Maleki, Soheila J.; Rabjohn, Patrick A.; Shin, David S.; Stanley, J. Steven (USA). U.S. Pat. Appl. Publ. US 2003202980 A1 20031030, 194 pp., Cont.-in-part of U.S. Ser. No. 494,096. (English). CODEN: USXXCO. APPLICATION: US 2002-100303 20020318. PRIORITY: US 95-PV9455; 19951229; US 96-717933; 19960923; US 98-PV73283; 19980131; US 98-PV74633; 19980213; US 98-PV74624; 19980213; US 98-PV74590; 19980213; US 98-106872; 19980629; US 98-141220; 19980827; US 98-191593; 19981113; US 99-241101; 19990129; US 99-240557; 19990129; US 99-248674; 19990211; US 99-248673; 19990211; US 99-PV122560; 19990302; US 99-PV122565; 19990302; US 99-PV122566; 19990302; US 99-PV122450; 19990302; US 99-PV122452; 19990302; US 99-267719; 19990311; US 2000-494096; 20000128.

AB It has been determined that allergens, which are characterized by both humoral (IgE) and cellular (T-cell) binding sites, can be modified to be less allergenic by modifying the IgE binding sites. The IgE binding sites can be converted to non-IgE binding sites by altering as little as a single amino acid within the protein, preferably a hydrophobic residue towards the center of the IgE epitope, to eliminate IgE binding. Addnl. or alternatively a modified allergen with reduced IgE binding may be prepared by disrupting one or more of the disulfide bonds that are present in the natural allergen. The disulfide bonds may be disrupted chemical, e.g., by reduction and alkylation or by mutating one or more cysteine residues present in the primary amino acid sequence of the natural allergen. In certain embodiments, modified allergens are prepared by both altering one or more linear IgE epitopes and disrupting one or more disulfide bonds of the natural allergen. In certain embodiments, the methods of the present invention allow allergens to be modified while retaining the ability of the protein to activate T-cells, and, in some embodiments by not significantly altering or decreasing IgG binding capacity. The immunotherapeutics can be prepared in transgenic plants or animals; and administered in injection, aerosol, sublingual or topical form. The immunotherapeutics can also be encoded in gene for gene therapy and delivered by injecting into muscle or skin to induce tolerance. The Examples provided herein use **peanut allergens** to illustrate applications of the invention.

L31 ANSWER 2 OF 4 CAPLUS COPYRIGHT 2005 ACS on STN
2003:906632 Correction of: 2002:736063 Document No. 139:349665 Correction of: 137:277814 Modified anaphylactic food allergens with reduced IgE-binding ability for decreasing clinical reaction to allergy. **Caplan, Michael**; Sosin, Howard; Sampson, Hugh; Bannon, Gary A.; Burks, Wesley A.; Cockrell, Gael; Compadre, Cesar M.; Connaughton, Cathie; Helm, Ricki M.; King, Nina E.; Kopper, Randall A.; Maleki, Sohelia J.; Rabjohn, Patrick A.; Shin, David S.; Stanley, J. Steven (Panacea Pharmaceuticals, USA; et al.). PCT Int. Appl. WO 2002074250 A2 20020926, 299 pp. DESIGNATED STATES: W: AE, AG, AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, BZ, CA, CH, CN, CO, CR, CU, CZ, DE, DK, DM, DZ, EC, EE, ES, FI, GB, GD, GE, GH, GM, HR, HU, ID, IL, IN, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MA, MD, MG, MK, MN, MW, MX, MZ, NO, NZ, OM, PH, PL, PT, RO, RU, SD, SE, SG, SI, SK, SL, TJ, TM, TN, TR, TT, TZ, UA, UG, US, UZ, VN, YU, ZA, ZM, ZW; RW: AT, BE, BF, BJ, CF, CG, CH, CI, CM, CY, DE, DK, ES, FI, FR, GA, GB, GR, IE, IT, LU, MC, ML, MR, NE, NL, PT, SE, SN, TD, TG, TR. (English). CODEN: PIXXD2. APPLICATION: WO 2002-US9108 20020318. PRIORITY: US 2001-PV276822 20010316.

AB It has been determined that allergens, which are characterized by both humoral (IgE) and cellular (T-cell) binding sites, can be modified to be less allergenic by modifying the IgE binding sites. The IgE binding sites can

be converted to non-IgE binding sites by altering as little as a single amino acid within the protein, preferably a hydrophobic residue towards the center of the IgE epitope, to eliminate IgE binding. Addnl. or alternatively a modified allergen with reduced IgE binding may be prepared by disrupting one or more of the disulfide bonds that are present in the natural allergen. The disulfide bonds may be disrupted chemical, e.g., by reduction and alkylation or by mutating one or more cysteine residues present in the primary amino acid sequence of the natural allergen. In certain embodiments, modified allergens are prepared by both altering one or more linear IgE epitopes and disrupting one or more disulfide bonds of the natural allergen. In certain embodiments, the methods of the present invention allow allergens to be modified while retaining the ability of the protein to activate T-cells, and, in some embodiments by not significantly altering or decreasing IgG binding capacity. The Examples provided herein use **peanut allergens** to illustrate applications of the invention.

L31 ANSWER 3 OF 4 CAPLUS COPYRIGHT 2005 ACS on STN
2001:416973 Document No. 135:45198 Prevention of an anaphylactic response to food allergens. Bannon, Gary A.; Burks, Wesley A.; **Caplan, Michael J.**; Sampson, Hugh; Sosin, Howard (Panacea Pharmaceuticals, LLC, USA; University of Arkansas; Mount Sinai School of Medicine, University of New York). PCT Int. Appl. WO 2001040264 A2 20010607, 100 pp. DESIGNATED STATES: W: AE, AG, AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, BZ, CA, CH, CN, CR, CU, CZ, DE, DK, DM, DZ, EE, ES, FI, GB, GD, GE, GH, GM, HR, HU, ID, IL, IN, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MA, MD, MG, MK, MN, MW, MX, MZ, NO, NZ, PL, PT, RO, RU, SD, SE, SG, SI, SK, SL, TJ, TM, TR, TT, TZ, UA, UG, UZ, VN, YU, ZA, ZW, AM, AZ, BY, KG, KZ, MD, RU, TJ, TM; RW: AT, BE, BF, BJ, CF, CG, CH, CI, CM, CY, DE, DK, ES, FI, FR, GA, GB, GR, IE, IT, LU, MC, ML, MR, NE, NL, PT, SE, SN, TD, TG, TR. (English). CODEN: PIXXD2. APPLICATION: WO 2000-US33124 20001206. PRIORITY: US 1999-455294 19991206; US 2000-PV213765 20000623; US 2000-PV235797 20000927.

AB The authors disclose methods for reducing allergic responses in individuals sensitive to one or more food antigens. In general, desensitization is achieved by administration of fragments of antigens characterized by a reduced ability to bind to their cognate IgE. In one example, mice were sensitized to **peanut allergens** by intragastric feeding. Administration of peptide fragments of Ara h 2, or an allergen mutein with altered IgE binding sites, abrogated an increase in IgE levels and anaphylactic sequelae.

L31 ANSWER 4 OF 4 CAPLUS COPYRIGHT 2005 ACS on STN
2001:416792 Document No. 135:10056 Controlled delivery of antigens. **Caplan, Michael**; Burks, Wesley A., Jr.; Bannon, Gary A. (The Board of Trustees of the University of Arkansas, USA; Panacea Pharmaceuticals, LLC). PCT Int. Appl. WO 2001039800 A2 20010607, 34 pp. DESIGNATED STATES: W: AE, AG, AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, BZ, CA, CH, CN, CR, CU, CZ, DE, DK, DM, DZ, EE, ES, FI, GB, GD, GE, GH, GM, HR, HU, ID, IL, IN, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MA, MD, MG, MK, MN, MW, MX, MZ, NO, NZ, PL, PT, RO, RU, SD, SE, SG, SI, SK, SL, TJ, TM, TR, TT, TZ, UA, UG, UZ, VN, YU, ZA, ZW, AM, AZ, BY, KG, KZ, MD, RU, TJ, TM; RW: AT, BE, BF, BJ, CF, CG, CH, CI, CM, CY, DE, DK, ES, FI, FR, GA, GB, GR, IE, IT, LU, MC, ML, MR, NE, NL, PT, SE, SN, TD, TG, TR. (English). CODEN: PIXXD2. APPLICATION: WO 2000-US42607 20001206. PRIORITY: US 1999-PV169330 19991206.

AB Formulations and methods are developed for delivering antigens to individuals in a manner that substantially reduces contact between the antigen and IgE receptors displayed on the surfaces of cells involved in mediating allergic responses, which target delivery of antigen to dendritic, phagocytic and antigen presenting cells (APCs), and which have improved pharmacokinetics. By reducing direct and indirect association of antigens with antigen-specific IgE antibodies, the risk of an allergic reaction, possibly anaphylactic shock, is reduced or eliminated. Particularly preferred antigens are those that may elicit anaphylaxis in

individuals, including food antigens, insect venom and rubber-related antigens. In the preferred embodiments, the compns. include one or more antigens in a delivery material such as a polymer, in the form of particles or a gel, or lipid vesicles or liposomes, any of which can be stabilized or targeted to enhance delivery. Preferably, the antigen is surrounded by the encapsulation material. Alternatively or addnl., the antigen is displayed on the surface of the encapsulation material. One result of encapsulating antigen is the reduction in association with antigen-specific IgE antibodies. In some embodiments, antigens are stabilized or protected from degradation until the antigen can be recognized and endocytized by APCs which are involved in eliciting cellular and humoral immune responses. In a preferred embodiment, the formulation is designed to deliver antigens to individuals in a manner designed to promote a Th1-type mediated immune response and/or in a manner designed to suppress a Th2 response. In still another embodiment, the formulation effects preferential release of the antigen within APCs. For example, various synthetic, biodegradable polymeric microsphere formulations were prepared containing **peanut allergen.** Microspheres based on poly(lactide-co-glycolide) (75:25) containing an acid end group (0.1% loaded with allergen) had the lowest amount (<20 ng) of peanut protein detected on the outside of the microsphere and the best range of peanut protein allergens contained within the microspheres (having mol. wts. ranging from 15 kDa to 70 kDa).

=>

---Logging off of STN---

=>
Executing the logoff script...

=> LOG Y

COST IN U.S. DOLLARS	SINCE FILE ENTRY	TOTAL SESSION
FULL ESTIMATED COST	303.80	304.01

DISCOUNT AMOUNTS (FOR QUALIFYING ACCOUNTS)	SINCE FILE ENTRY	TOTAL SESSION
CA SUBSCRIBER PRICE	-29.20	-29.20

STN INTERNATIONAL LOGOFF AT 16:50:47 ON 18 APR 2005